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NOTES ON THE BIOLOGY OF THE COPEPOD *MYTILICOLA INTESTINALIS* STEUER

BY J. N. R. GRAINGER, M.SC., *From the Department of Zoology, University College, Hull*

(With 6 Figures in the Text)

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INTRODUCTION

Mytilicola intestinalis Steuer (1902, 1905) is a parasitic copepod belonging to the family Clausiidae, and is found in the gut of molluscs of the genus *Mytilus*. The second antennae are prominent and hook-like and enable the copepod to cling to the gut wall of the mussel. The legs are reduced, and flaps have been developed on the dorsal side of the body. The copepods move in worm-like fashion when removed from their host. The mouthparts are reduced and, as Monod & Dollfus (1932) have pointed out, the mandibles seem to have disappeared. The red colour of the adults is due to haemoglobin (Ellenby, 1947). The colour gradually increases in intensity after the first adult stage. The sexes differ considerably in size in the adult; the male is about 4 mm. and the female about 8 mm. in length. The free-living stages (nauplius, metanauplius and first copepodid) have been described by Pesta (1907). The first two parasitic stages (second and third copepodid) have been described by Caspers (1939) and the next, that is the first adult stage, by Pesta (1907). Subsequent development has not been described in detail.

The genus *Mytilicola* appears to be confined to the Northern Hemisphere. *M. ostrea* was described by Wilson (1938) from Japanese oysters (*Ostrea gigas*) which had been transplanted to the Pacific coast of North America but this species has not apparently been recorded from Japan. Mori (1935), however, described *M. orientalis* from the gut of *Mytilus crassitesta* and also *Ostrea gigas* which were collected in the Inland Sea of Japan.

Mytilicola intestinalis was first described by Steuer (1902, 1905) from *Mytilus galloprovincialis* collected at Trieste and Gravosa. Since then there have been a number of records from the Mediterranean, which have been summarized by Monod & Dollfus (1932) and Caspers (1939). There is apparently only one record of the copepod from *M. edulis* in the Mediterranean (Vayssière, 1914). Outside the Mediterranean the copepod has only been reported from *M. edulis*. Caspers (1939) recorded it for the first time from the North Sea, where he found infected mussels at several localities between Cuxhaven and Neuharlingersiel. Ellenby (1947) reported it from the River Blyth, Northumberland. This, however, is not apparently the first record from the British Isles, since there is a specimen in the British Museum collection which was taken at Portsmouth in 1937.

NOTES ON LIFE CYCLE

The first three stages in the life cycle (nauplius, metanauplius and first copepodid) are free-swimming. The ripe egg-sacs on the female *Mytilicola intestinalis* fracture at their posterior extremity and the nauplii, each enclosed by a membrane, are shed into the gut cavity of the mussel. It is not known when the nauplii emerge from the membrane, but in sea-water cultures they hatch direct from the egg-sac and leave the membrane behind.

At laboratory temperatures the nauplius is positively phototropic and very active. The metanauplius is less active and has a weaker phototropic response. The first copepodid is, like the nauplius, an active swimmer, but individuals differ considerably in their response to light; some have a positive, some a negative, and some scarcely any response to ordinary daylight. These stages were reared successfully from detached egg-sacs in Petri dishes containing sea water. No difficulty was experienced in keeping the larvae alive provided there was no overcrowding. When crowded there were numerous deaths, especially at the metanauplius stage. Ten to twenty individuals in a 4½ in. Petri dish was found very satisfactory and no aeration was necessary. At 13–14° C. ten larvae had all reached the first copepodid stage within 40 hr. after hatching. They lived for up to 11 days after reaching this stage. Other

experiments showed that at 8–9° C. development is considerably retarded, and the larvae did not appear to be so active.

When kept in sea water which had been passed through a Berkefeld filter to remove diatoms, etc., the free-living stages appeared to develop normally. This, together with the fact that the mouthparts are somewhat reduced, suggests that these stages are independent of external food and can exist on their internal yolk supply until they reach a mussel. If a first copepodid failed to reach a mussel it died without undergoing any further moults. This confirms a similar statement by Caspers (1939). It is doubtful if nauplii or metanauplii are capable of infecting mussels, since these stages, unlike the first copepodid or later stages, have no prominent hook-like appendages to enable them to cling to the gut wall. The mode of entry of the first copepodids into the mussel is not known.

An experiment was carried out which gives approximate figures for the duration of the early parasitic stages in the mussel. Six batches, each of five uninfected mussels, were infected with first copepodids and kept in filtered aerated sea water at about 15° C. The first copepodids used in the experiment had moulted to this stage not more than 24 hr. previously. In order to facilitate subsequent detection of the larvae the mussels were kept in filtered sea water for a short time before the experiment. This removed much of the detritus from the gut. The mussels were examined at intervals of 2, 4, 8, 12 and 20 days. On the 12th day all the larvae found in the gut had moulted to the second copepodid stage and on the 15th day the majority were third copepodids. On the 20th day after infection all the larvae found had reached the third copepodid stage except one which had moulted to the first adult.

Females can be distinguished at 2·5 mm. when the chitinous oviduct openings are clearly visible. In the male the testes can be distinguished in 2 mm. larvae.

Fertilization has not been observed, but presumably takes place in the gut of the host, since the gonads are scarcely differentiated in the last free-swimming stage. The first adult and all later stages cannot swim because of the reduced appendages, and so if they leave a mussel it is doubtful if they are able to infect another.

DISTRIBUTION IN IRELAND

Samples of mussels were examined from twenty-one localities in Ireland. The location of each sample is shown in Fig. 1. *Mytilicola* was found in four localities (Fig. 1; Table 1). One of these (sample 6) was in Co. Galway and the other three (samples 1–3) in Co. Cork. It was found that the larger mussels were the most heavily infected (Fig. 4). For this reason each sample chosen consisted of the larger mussels from each bed. All the samples were collected between January and September 1948, and, with a few exceptions, each consisted of twenty mussels. Two of the four samples which were infected with *Mytilicola* (samples 2 and 3) were from estuaries. Sample 1 was not from an estuary but the salinity of the water was low (29°/$_{oo}$ when the mussels were covered at high tide on 29 January 1948), owing to the presence of a large estuary nearby. Sample 6 also was not from an estuary, but the salinity of the water was not determined so the mussels cannot be regarded with certainty as coming from a strictly marine bed. The four infected samples were all from the neighbourhood of shipping ports.

Caspers (1939) found that mussels collected at Neuwerk, Karolinensiel and Neuharlingersiel in the North Sea contained a mean number of 8·1, 7·2 and 5·7 *Mytilicola* per mussel respectively (calculated from Caspers's tables). Since, however, Caspers does not state the size of the mussels which he examined, it is difficult to make an accurate comparison with the present data. With the exception of sample 6 the number of parasites per mussel examined appears to be lower than was found by Caspers. The largest number of *Mytilicola* found in a single mussel was 27. The mussel was 65 mm. in length and was taken in sample 6. The twenty-seven copepods consisted of fourteen adult males, nine adult females and four immature stages.

ECOLOGICAL SURVEY OF AN INFECTED MUSSEL BED

Methods

A survey was carried out from November 1947 to October 1948 of a *Mytilicola* population in an infected *Mytilus edulis* bed at Fountainstown, Co. Cork. The bed is situated in the mouth of a small estuary and is exposed to the air for a short time at most low tides. Samples of mussels of various sizes were collected from all parts of the bed each month, except March 1948 (Fig. 3). The majority of the mussels in the bed were of length 50–70 mm. Mussels smaller than this size were scarce and had to be searched for. Each sample was examined alive soon after it was collected, except those taken in April and July which were preserved in 10 % formalin for a period before examination. A few mussels collected in November and February were not examined for up to 10 days after they were collected. During that time they were kept at 9° C. in a tank of aerated sea water. This may have lowered slightly the relative numbers of immature stages recorded for these months. No stages earlier than the first adult were found in any of these mussels so kept. Since at 15° C. it takes at least 20 days to reach this stage under laboratory conditions it is thus improbable that

any of the stages found originated as new infections from other mussels in the tank (see above).

Each mussel was examined after removal of recurrent intestine and rectum were dissected out under a binocular microscope. The stomach and direct intestine (Fig. 2, *DI*) were opened and ex-

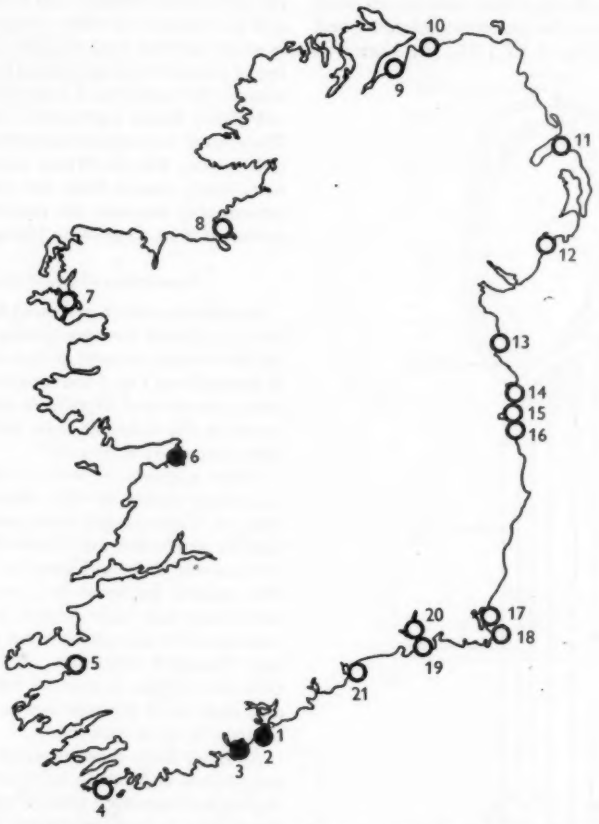

Fig. 1. Map showing the location of the samples of *Mytilus edulis*. Black circles indicate infection with *Mytilicola intestinalis*; white circles indicate that no *Mytilicola* were found.

Table 1. *Details of samples of* Mytilus edulis *infected with* Mytilicola intestinalis

Sample reference no.	Locality	Date	Size of sample	Mean length of mussels (mm.)	Mean no. *Mytilicola* per mussel
1	Church Bay, Crosshaven, Co. Cork	16 Jan. 1948 and 4 Feb. 1948	20	26·0	1·1
2	Fountainstown, Co. Cork		See 'Survey'		
3	River Bandon, Kinsale, Co. Cork	12 Mar. 1948	10	70·9	1·7
6	Kinvara, Co. Galway	20 Mar. 1948	10	61·8	7·7

one valve by cutting the attached muscles. The length of the mussel was measured to the nearest millimetre. The entire viscera were removed and the amined *in situ*. The position, sex and approximate length of each *Mytilicola* found were noted. No copepodid stages were found in any of the mussels

examined in the survey. The term 'immature stages', therefore, refers to all later stages except the final adult.

Distribution in mussel gut

All the specimens of *M. intestinalis* which were found were taken from the recurrent intestine and rectum of the mussel (Fig. 2, RI_1, RI_2, R). None was found in the rest of the alimentary canal (Table 2). Most of the copepods occurred in the region of the recurrent intestine which is embedded in the digestive gland (Fig. 2, RI_2). [In some other work a small number of mussels were examined which had been kept in badly aerated sea water. There appears to be a tendency for the *Mytilicola* in these to move down the gut (Table 2). The activity of the copepods did not appear to have been diminished by the poor aeration.]

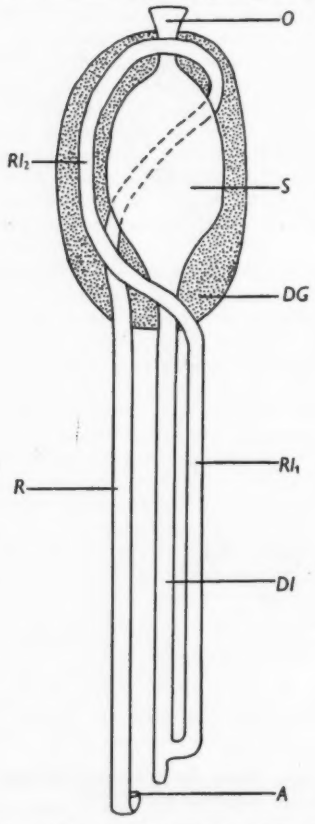

Fig. 2. Alimentary canal of *Mytilus edulis* (diagrammatic). *A*, anus; *DG*, digestive gland; *DI*, direct intestine; *O*, oesophagus; *R*, rectum; RI_1 and RI_2, recurrent intestine; *S*, stomach. (Partly from White, 1937.)

Size of mussel and number of Mytilicola *per mussel*

There is a significant* correlation (correlation coefficients = 0·427 and 0·628 for the two periods May to June and November to December respectively) between mussel length and mean number of *Mytilicola* per mussel collected—larger mussels being more heavily infected than smaller ones. The mean number of parasites per *Mytilus* is lower in May and June than in November and December. Fig. 4 shows the calculated linear regressions for these two periods. They differ very significantly ($t = 4{\cdot}44$ for 111 degrees of freedom). The 20–29 mm. mussel group was almost completely absent from the bed in May and June, presumably because the mussel spat did not have sufficient time to grow to this size.

Population changes during the year

In order to obtain sufficient data for an analysis of the population changes during the year the 50–59 and 60–69 mm. mussel groups were treated together. It is seen from Fig. 5 that despite the variation in the mean numbers of *Mytilicola* per mussel, these were lower in the summer than in winter. No mussels were examined in March.

There appears to be a cycle in the numbers of immature stages in the mussels during the year (Fig. 5). These stages were present in highest numbers in November and December. None was found in June and July and very few in May and August. The mussels gathered in April and July were preserved, as has been stated, and when they were subsequently examined some of the parasites were in a damaged condition. It is possible, therefore, that the smaller immature forms were overlooked, and this could perhaps account for their apparent absence in these months.

Judging from these samples the adult *Mytilicola* population appears to have reached a minimum in September, at which time it was being reinforced by developing immature stages. The adult population was apparently highest from November to February but the height of the February column (Fig. 5) may not be significant as only a few mussels were examined in this month.

There was an unexpected excess of adult males in the samples throughout the year (Fig. 6). Of all the adults examined in the year from the 50–69 mm. mussel group 64·0 % were males. It is doubtful if the undulating character of Fig. 6 is significant, as the numbers of copepods examined in some months were small. None was examined in March. When the adult population was lowest in September the numbers of the sexes were very unequal (73·5 % male).

Females carrying egg-sacs were found throughout the year, but more carried them in the summer (Table 3).

* The level of significance used throughout is $P < 0{\cdot}01$.

Table 2. *Position of* Mytilicola intestinalis *in gut of* Mytilus edulis

	Fresh mussels		Poorly aerated mussels	
	No. of *Mytilicola*	*Mytilicola* (%)	No. of *Mytilicola*	*Mytilicola* (%)
Stomach and oesophagus	—	0·0	—	0·0
Recurrent intestine (Fig. 2, RI_1)	110	11·2	1	1·6
Recurrent intestine in digestive gland (Fig. 2, RI_2)	709	72·0	36	60·0
Rectum	119	12·1	20	33·3
Position uncertain	47	4·8	3	5·0
Total	985	100·0	60	99·9

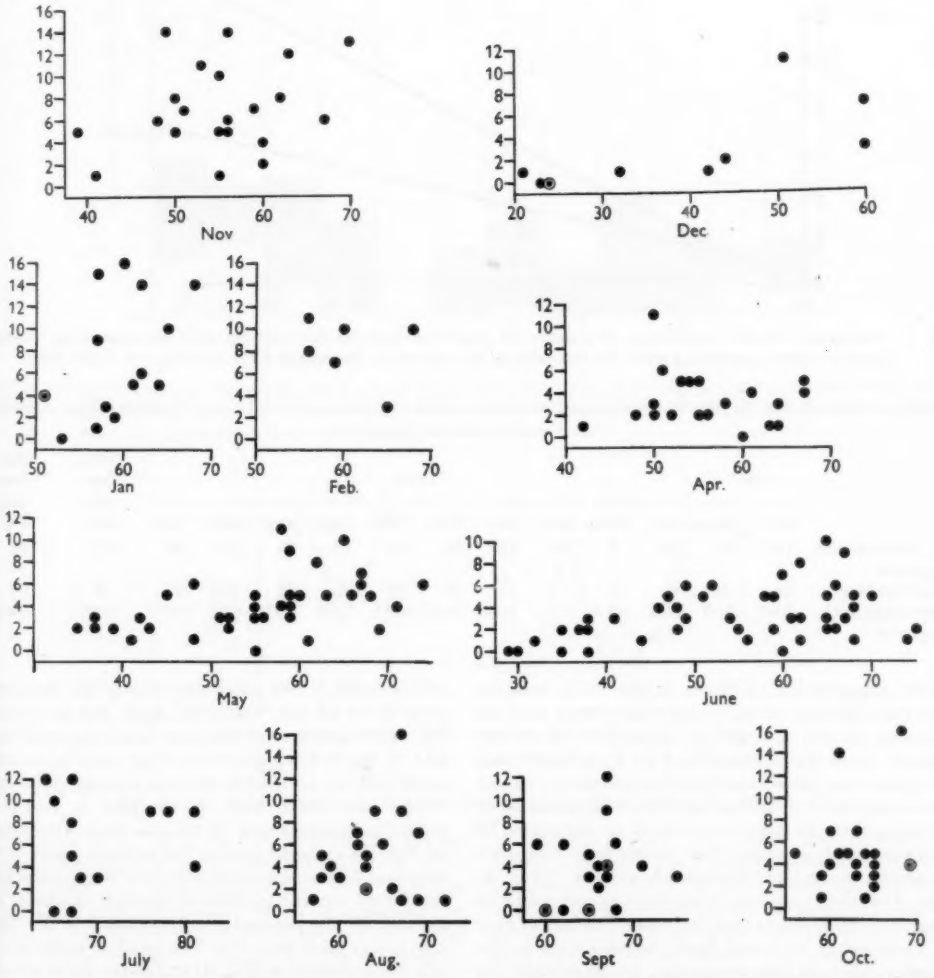

Fig. 3. Infection of *Mytilus edulis* examined in the survey with *Mytilicola intestinalis*. Number of *Mytilicola* on vertical axis; length of *Mytilus* (mm.) on horizontal axis. Dots with a circle indicate two dots occupying the same position.

DISCUSSION

Mytilicola intestinalis has not apparently been reported from all parts of the geographical ranges of *Mytilus edulis* and *M. galloprovincialis*, and so far has only been recorded from the Mediterranean, North Sea and British Isles (see Introduction). Caspers (1939) and Ellenby (1947) have noted that the records of the parasite appear to be from estuarine areas. With the exception of two samples this also appears to be the case in Ireland (p. 136). Caspers (1939) suggested that there might be a relation between pollution of the water with sewage and the presence of the copepod in estuaries. However, mussels from the infected bed at Fountainstown, Co. Cork, were tested and found to contain no excessive numbers of coliform bacteria. The presence of these bacteria in large numbers is an indication of pollution with sewage. This shows that Caspers's suggestion is not valid, in this case at least. Dr H. A. Cole (1948) has suggested that the copepod might be carried in mussels on ships' bottoms. The larvae may be liberated in port and infect mussel beds in the vicinity. This, as Cole points out, would explain the fact that all the mussel beds from which the copepod has been reported are apparently estuarine and in the neighbourhood of shipping ports. The marine beds, on the other hand, which have so far been examined in the British Isles and North Sea (Fig. 1, also Caspers (1939), Ellenby (1947)) are apparently uninfected. It would be of interest to find out if the marine beds are also free from infection in the Mediterranean where the copepod has probably been present for longer.

The rate of mussel growth was not studied in the bed examined in the survey. In the Plymouth district, where the rate of growth is apparently rapid, White (1937, p. 96) notes that young *M. edulis* may grow to 32–38 mm.* between April and November. The next April the mussels may be as long as 51 mm., and by the following October they may be as much as 57–83 mm. in length, but the average is 51 mm. White also notes that in England a 4-year-old mussel is usually about 79–89 mm. long. The growth of *Mytilus* is rapid during the summer, and so the numbers in any size group are then being constantly reinforced by the growth of smaller mussels, and reduced by the passage of individuals into the larger size group next above it. The smaller mussels contain fewer copepods (Fig. 4) and so the mean number

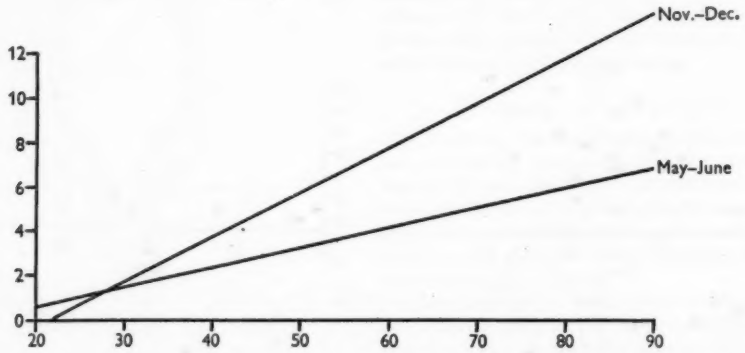

Fig. 4. Calculated linear regressions of number of *Mytilicola intestinalis* (vertical axis) per mussel on length of *Mytilus edulis* (horizontal axis) for the periods November to December 1947 and May to June 1948.

Table 3. *Occurrence of female* Mytilicola intestinalis *with egg-sacs in* 50–69 mm. Mytilus edulis *collected at Fountainstown, Co. Cork*

	1947		1948										Nov., Dec. and Jan.	May, June and July
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.		
No. females with egg-sacs	19	2	16	7	—	19	33	18	17	23	9	13	37	68
Total females	34	4	36	12	—	27	47	29	22	36	13	25	74	98
Percentage with egg sacs	55·9	50·0	44·4	58·3	—	70·4	70·2	62·1	77·3	63·9	69·2	52·0	50·0	69·4

* I have converted White's (1937) figures from inches to millimetres.

of *Mytilicola* per mussel for all size groups except the largest one is lowered, since new infection is almost zero from April to August (Fig. 5). This gives an exaggerated view (Fig. 5) of a real decrease in the copepod population. Fig. 4 shows that in order to have the same number of *Mytilicola* a 55 mm. November mussel would, on the average, have to

lation is being constantly reinforced. In mussels smaller than 55 mm. it is doubtful if there has been a real decrease (Fig. 4).

Egg-bearing *Mytilicola* were found throughout the year (Table 3). There appears to be an increase in the number bearing egg-sacs in the summer. It is not unlikely that the eggs laid in the autumn are

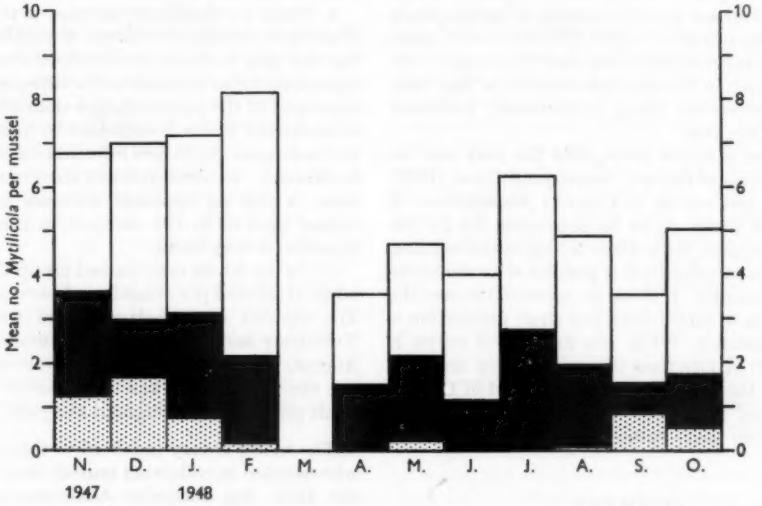

Fig. 5. Diagram showing the composition of the *Mytilicola* population in 50–69 mm. mussels taken from the bed examined in the survey from November 1947 to October 1948. Adult females black, adult males white, immature stages stippled. No mussels were examined in March.

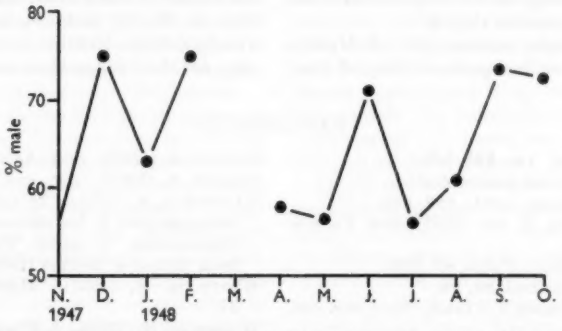

Fig. 6. Sex ratio in adult *Mytilicola intestinalis* taken from 50 to 69 mm. mussels.

grow to 90 mm. by June. It is therefore almost certain (unless the rate of growth of the *Mytilus* in the bed studied was exceedingly rapid) that there has been a real decrease in the numbers of *Mytilicola* per mussel between November–December and May–June in the larger mussels (> 55 mm.) at least. The death-rate of the parasites is probably still higher than would appear from these considerations, since in the months November to February the *Mytilicola* popu-

carried throughout the winter and develop very slowly because of the lower temperatures. When the temperature rises in the summer the eggs may hatch to produce larvae which enter the mussels in numbers in the autumn. Those larvae entering in late autumn may develop rather slowly and gradually become adult during the winter. This would account for the low numbers of immature stages recorded in May and June and the larger numbers found from Sep-

tember to January (Fig. 5). It should be noted, however, that the low numbers of immature stages recorded in the summer months may be due to other causes (e.g. large numbers of predators attacking the free-living stages) or they may not occur in all years. Sproston & Hartley (1941), working on other copepods, noted that with *Clavella uncinata* 'reproduction takes place throughout the year, though since conditions are more favourable at certain times the main recruitment to the *Clavella* stock takes place in the autumn in the sea near Plymouth'. On the other hand, in *Lernaeocera branchialis* they note that generations are being continuously produced throughout the year.

The excess of males throughout the year may be due to a number of factors. Raymont & Gross (1942) found that the excess in *Calanus finmarchicus* of females over males could be accounted for by the fact that the males did not live as long as the females. In *Mytilicola intestinalis* it is possible that the males outlive the females. If this is so one would expect the largest excess of males when the adult population is low in September. While the number of males is high (Fig. 6) at this time this explanation does not account for the high numbers also found in December, February and June. Much more data will be needed before any definite conclusions can be drawn.

SUMMARY

1. The free-swimming larvae of *Mytilicola intestinalis* can develop in the absence of external food. Approximate figures are given for the duration of the free-living and early parasitic stages.

2. Four of the twenty-one samples of *Mytilus edulis* collected from various parts of Ireland were infected with *Mytilicola intestinalis*. All the four mussel beds found to be infected were in the neighbourhood of ports.

3. 72·0 % of the *Mytilicola* which were found inhabited the part of the recurrent intestine which is embedded in the digestive gland.

4. There is a significant correlation between mussel length and number of *Mytilicola* per mussel.

5. There is a significant decrease in the numbers of *Mytilicola* per mussel between November to December and May to June. It is almost certain that this represents a real decrease in the copepod population, especially in the mussels larger than 55 mm., due to a death-rate which is sufficient to more than offset the increment due to new infection during November to January. In certain chosen size groups of mussels there is also an apparent decrease as a result of mussel growth in the summer, a time when new infection is very small.

6. In the 50–69 mm. mussel group the number of adult *Mytilicola* per mussel was lowest in September. The number of immature stages was highest in November and December and lowest from May to August. Egg-bearing copepods were found throughout the year. There was an excess of males in the adult population throughout the year.

The writer is very indebted to the various people who assisted in collecting mussel samples especially the Irish Sea Fisheries Association; to Miss M. Davidson, B.A., who made a bacteriological examination of a mussel sample; to Dr J. P. Harding for informing him of the specimens of *Mytilicola intestinalis* in the British Museum collection; and to Prof. J. Brontë Gatenby and Dr J. D. Smyth of Trinity College, Dublin (where this work was carried out), for their suggestions and advice.

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LERNAEA CHACKOENSIS N.SP.: A COPEPOD PARASITIC ON TWO MADRAS FISHES

By C. P. GNANAMUTHU, *Director, Zoology Research Laboratory, Madras University*

(With 8 Figures in the Text)

Since 1925, when Leigh-Sharpe described *Lernaea elegans*, the first Asiatic species from *Anguilla japonica*, four more species were recorded from China and Japan. The species described in the present paper, while differing from these and other known species, forms the first record of the genus from India. Five parasites attached to three fishes (*Osphronemus goramy*) and twelve removed from their hosts, together with two fish (*Catla catla*) infested by about eighteen parasites formed the material examined. Colonies of a vorticellid (*Carchesium* sp.) were found attached to the external portions of the parasites. In some the stalks of the epizoans were woven through by Algae such as *Oscillaria*, *Lyngbya* and *Calothrix*, enmeshing other algae like *Cosmaria*, *Oedogonium* and diatoms, and a matted covering was thus formed round the bodies of the parasites.

Size and colour. The total length of the adults varied from 6 to 8 mm. exclusive of the egg-sacs and anal laminae. In a form measuring 7 mm. the head was 0·24 mm., the first segment 0·4 mm., the free thorax 2·2 mm., the trunk 3·36 mm. and the abdomen 0·8 mm. The egg-sacs varied, even in the same female, from 1·6 to 2 mm. and contained five to six rows of about twenty to twenty-five eggs each. The parasites were transparent with a slight greenish tinge, and the egg-sacs greenish or brownish. Formalin preserved specimens were dark brown.

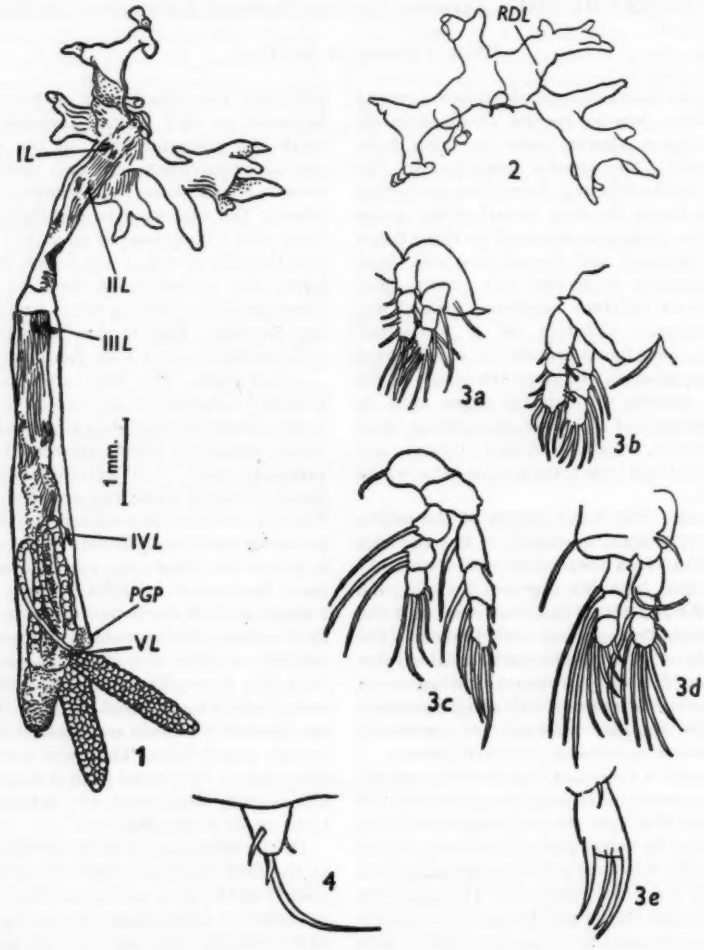
Body. The head is a circular lobe showing several slender radiating columns of muscles and hoods over the mouthparts. The first thoracic segment, fused to the head, is the broadest part of the body, and is extended laterally into four arms appearing like an X when viewed from the front end. The arms are simple in the young forms and become completely branched and asymmetrical in the adult. This heterogonic growth and branching of the arms is particularly noticeable in the parasites taken from *Catla catla* (Fig. 8). The posterior region of the first segment bears the first pair of legs between the two ventro-lateral arms. The neck or free thorax is uniformly slender, cylindrical, nearly two-thirds the trunk in length. A few individuals exhibited a distension about the middle. It bears the second and third pairs of legs. Behind the third pair starts the trunk, the longest part of the body. Anteriorly, it is as slender as the neck, but gradually thickens to form the genital region and bears the fourth and fifth legs. The attachment of the abdomen and the egg-sacs, as well as the pregenital prominences, mark the posterior limit of the trunk. The pregenital prominences are two large swellings very conspicuous in some individuals but less marked in others. The abdomen is a cylindrical blunt-ended rump of the body held at an angle. It is constricted into three in all the young forms, while in the older forms the constrictions tend to disappear. The truncated posterior tip bears two conical tapering anal laminae. Each lamina bears three short spines and terminates in a long ventrally curved seta.

Appendages. The first antenna is four-jointed, long and cylindrical, and can be seen extending on either side of the cephalic prominence. The proximal three segments bear sixteen hook-like spines ventrally, while the terminal segment bears ten sharp spines of which two are stout recurved claws. The second antenna is attached close to the first. It is shorter and three-jointed. The second bears a small spine on the distal edge and the long terminal segment bears seven hook-like spines. The mandible is a sharp curved blade having no teeth on its edges. It is usually hidden behind the maxillae. The first maxilla consists of a stout base bearing a narrow hook-like blade. The second maxilla is of two long stout curved claws fused together. The maxillipedes are directed forwards and pressed close to the other mouth appendages. They are stout, stumpy, and two-jointed. The basal joint is long, faintly divided transversely and bears two lateral clawed digitations on its distal edge.

Of the five pairs of thoracic legs, the first occurs in the first segment, while the remaining four are spaced on the neck and trunk, the distances varying in different individuals depending on the torsion of their bodies. The first four legs have a two-jointed protopod bearing a spine on the inner edge and two three-articled rami. The endopod of the first leg carries eight spines while the exopod has eleven. The second leg has nine spines on the endopod and twelve on the exopod. The third and fourth legs have eight spines on the endopod and twelve on the exopod. The fifth leg is vestigial, uniramous as in other Caligoids, and arises near the base of the egg-sac. Each consists of a basal papilla bearing a small spine, and a single distal joint bearing four longer spines.

Taxonomy. According to the key given by Wilson

(1917*a*) for the species of the genus *Lernaea* the present species differs from those he listed in that the pregenital prominence is divided and the lobes are not as long as the abdomen. Of the species founded present form differs from these in possessing two latero-dorsal and two ventral arms of equal size, as well as several differences in the appendages of the head and body. Taken together, with the fact of its



Figs. 1–4.

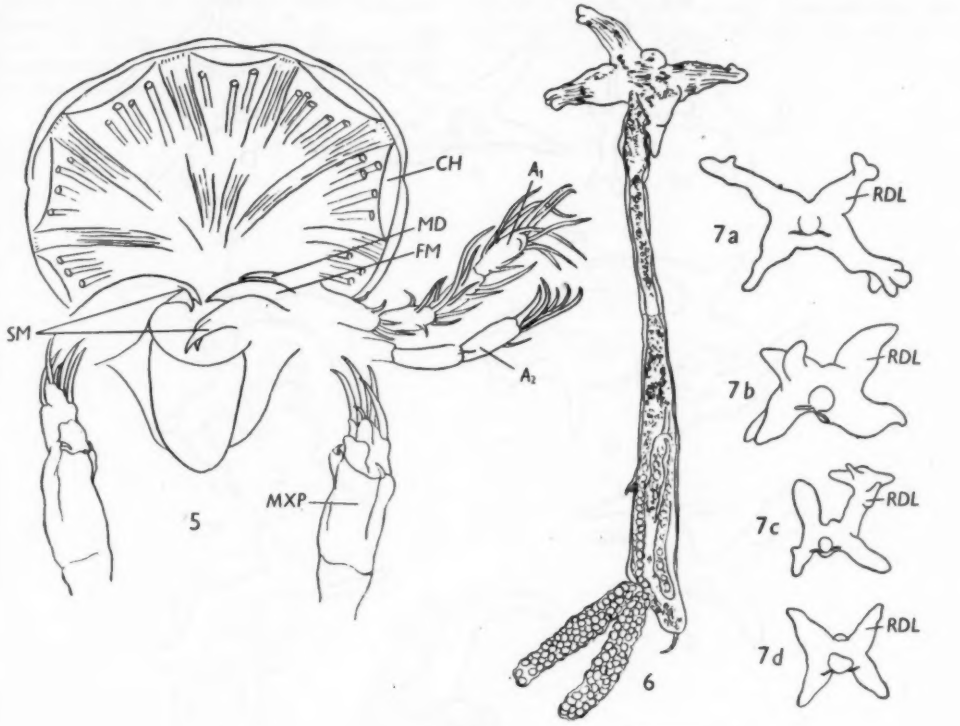
Fig. 1. Ventral view of *Lernaea chackoensis* n.sp. I *L*–V *L*, first to fifth leg. *PGP*, pregenital prominence.
Fig. 2. Anterior view of cephalic horns. *RDL*, right dorso-lateral arm.
Fig. 3. Thoracic appendages. *a*, *b*: I and II legs of right side; *c*, *d*, *e*: III, IV and V legs of left side.
Fig. 4. Anal lamina.

since 1917, *L. elegans* (Leigh-Sharpe, 1925), *L. composita* (Wilson, 1929), *L. barbicola* (Leigh-Sharpe, 1930), *L. ranae* (Stunkard, 1931), *L. carassi* (Tidd, 1933), *L. polymorpha*, *L. parasiluri*, *L. brachycera* (Yü, 1938), *L. parasiluri* (Yamaguti, 1939), *L. rhodei* (Hu, 1948) and three subspecies may be mentioned. The occurring on fresh-water fishes of the Indian region, it can be treated as a new species *L. chackoensis*.

Specific characters. Cephalic arms, four, equal, lateral; simple in young but branched and asymmetrical in adults. Head circular, hooding. Neck distinct. First antennae bear twenty-six spines,

second antennae seven spines on terminal joint. The maxillipedes bear seven claws. The form of the mandibles, the maxillae and the vestigial fifth leg, as well as the chaetotaxy of the legs, are characteristic. The pregenital prominence is divided into two rounded lobes. The anal lamina bears two outer and an inner spine as well as a long terminal spine.

hosts. In the young forms, the arms are simple, equal and symmetrical, but become asymmetrical, branching in a variety of ways. The degree of complexity attained by the species in the two different host environments also is noteworthy. These facts support Wilson's and Leigh-Sharpe's conclusions regarding the unreliability of the details of the form



Figs. 5–7.

Fig. 5. Head appendages. A_1, first antenna; A_2, second antenna; *CH*, cephalic hood; *FM*, first maxilla; *MD*, mandible; *MXP*, maxillipede; *SM*, second maxilla.

Fig. 6. Dorsal view of the parasite from *Osphronemus goramy*.

Fig. 7. Anterior views of cephalic arms of parasites taken from *Osphronemus goramy*. *a*, *b*, *c*, adults; *d*, young form.

Locality. Madras.

Hosts. *Osphronemus goramy* Lacépéde and *Catla catla* Cuv. & Val.

Type. The Holotype will be deposited in the Indian Museum, Calcutta.

REMARKS

There is considerable phenotypic variability in the configuration of the cephalic arms, as can be seen from the two sets of camera lucida drawings of the members of the species infesting the two different

of the arms, in determining species. Other features of the body also display considerable variability. The variations form a continuous series in which two ecotypes distinctive of the two different host environments can be recognized. The forms found on *Osphronemus goramy* have a more slender body, a relatively longer neck or free thorax, a shorter stouter abdomen constricted into three in the young. Those taken from *Catla catla* appear more stocky owing to the stouter shorter neck and greater width of the trunk and appear more variable in the number of branches, disposition and lateral

extension of the arms. These character gradients are more suggestive of a variable population than of two well-defined subspecies.

The type and degree of torsion in the twenty-three parasites studied *in situ* on their hosts, support Wilson's observations on *Lernaeenicus*. Whether the parasites are attached to exposed or sheltered parts, of the right or left side of the host, torsion appears to be inverse or direct, following no rule. The part of the body which is twisted also varies in the same way. The penetration of the young parasite through the host's tissues before reaching its final anchorage, as well as the necessity of the adult having to hold the egg-sacs away from the surface of the host's body, may be two factors jointly responsible for the torsion. Though the direction of the torsion may be a fortuitous circumstance, it is just possible that if the body twists in opposite directions owing to these two factors, the adult may show no torsion at all, while if they happen to cause a twist in the same direction, even a complete turn may result.

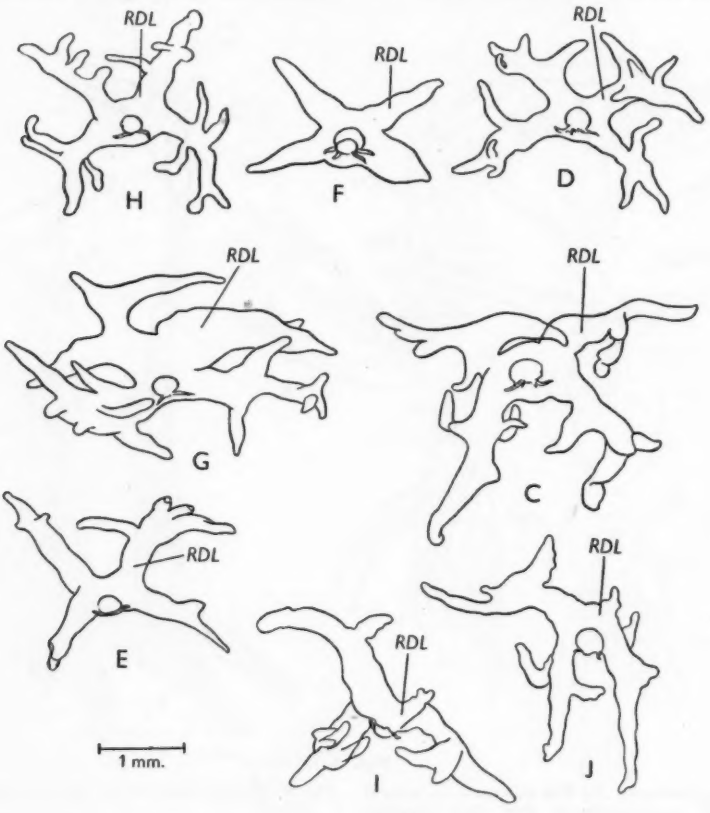


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Fig. 8. Anterior views of cephalic arms of eight (*C* to *J*) parasites taken from *Catla catla*. *RDL*, right dorso-lateral arm.

The author thanks Mr P. I. Chacko, M.A., Assistant Director of Fisheries (Freshwater Biology), Madras, for the gift of the material. The author is also indebted to Mr T. V. Desikachari, M.Sc., of the Botany Department for identifying the algae.

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NOTES ON THE LIFE HISTORY OF A PARASITIC COPEPOD, *LERNAEA CHACKOENSIS*

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(With 32 Figures in the Text)

The life history of the genus *Lernaea* was sketched by Wilson in 1917 *a* from different stages belonging to probably four different species. In 1927, Nabutaka Nakai described the development of *L. elegans* Leigh-Sharpe in great detail, but unfortunately, as his experimental infections failed, he had to describe the intermediate stages (i.e. second copepodid to the fifth) from different infected fish of *Cyprinus carpio* L., the host of the adult parasite. He was therefore not in a position to note the duration of each stage, and to say whether an intermediate host is necessary to complete the life cycle, as was held by Wilson. The present study of the development of this new South Indian species (Gnanamuthu, 1951), was undertaken because of the reported retarded growth of the infested *Osphronemus goramy* and *Catla catla*, and the desirability of protecting these food fishes. Since successful artificial infections threw light on the hosts and the duration of the intermediate stages, as well as on the earlier incidence of sex differentiation and the accelerated development of this tropical species, the present account was considered worth while.

MATERIAL AND METHOD

Preserved specimens of *Oryzias rubristigma*, *Gobius griseus*, *Catla catla*, as well as a few living *Osphronemus goramy* infested by the adult parasite, were obtained through the kindness of Mr P. I. Chacko, M.A., Assistant Director of Fisheries, Madras. The parasites were found to be different ecotypes of the same species *Lernaea chackoensis* n.sp. (1949). When a few heavily infested gourami were kept alive in glass troughs, it was found that the parasites were rubbed off the body, except in the region of the median fins. The egg-sacs of some were removed and the eggs were hatched *in vitro*. The fishes (*Oryzias rubristigma*, *Barbus stigma*, *Labeo calbasu* and *Cirrhina reba*), used for artificial infection experiments, were previously kept under observation for a few days. The laboratory temperature ranged between 77–79° F. at night and 90–94° F. during day through the entire period of study.

The eggs

Longitudinal and transverse sections of the body show that the genital protoplasm of the ovary is formed into egg filaments anteriorly. In the first limb of the oviduct, the egg-cells can be seen ripening but, owing to poor fixation, oogenesis and fertilization were difficult to follow. The eggs appear larger and darker in the second limb of the oviduct where the ducts of the seminal receptacle open. In the third or final limb, the eggs have a distinct vitelline membrane and are emptied into the glandular external passage called the cement gland (Wilson). Here the eggs acquire their thick, tough, covering, and are pushed into the external sacs through the vulvae. The eggs show a superficial layer of cytoplasm with a network of thin strands running into the interior which is packed with yolk globules. Ehrlich's haematoxylin renders the large eccentric nucleus and the nucleolus, conspicuous. The yolk globules differ in size, the smallest being towards the periphery, but even the larger ones were not flattened by contact with each other. The eggs in the external sac are about 0·132 mm. in diameter, more or less spherical, transparent, slightly greenish or brownish in colour. Those of the same egg-sac were not of the same age and hatched independently within a few minutes of each other.

Details of cleavage, and the formation of the germ layers, could not be made out in sections or in whole eggs, owing to faulty preservation; but the occurrence of three pairs of pads ('Kopfplatten') and the absence of a blastodermic cuticle are clear. When egg-sacs plucked from the parasites were left in filtered tap water at the laboratory temperature 90–94° F., the eggs hatched within 1 or 2 days.

The nauplius

When just hatched, the larva has an elliptical body 0·186 mm. long, exclusive of the balancers, and 0·13 mm. broad and grows more in length than breadth. In older larvae the hind-end becomes more acutely rounded than the broader frontal edge and the body becomes oval in outline. A distinct, X-shaped crimson pigment spot, close to the front border, further distinguishes the nauplius of this species from those described by Nakai and Wilson. Though slightly greenish in colour, the body is transparent, filled with yolk globules and teloblastic cells, which obscure the gut to such an extent that only faint limits can be made out by focusing. The dorsal shield is convex above, whereas the ventral shield is flatter. Between these, at the posterior end, two short spine-like balancers are

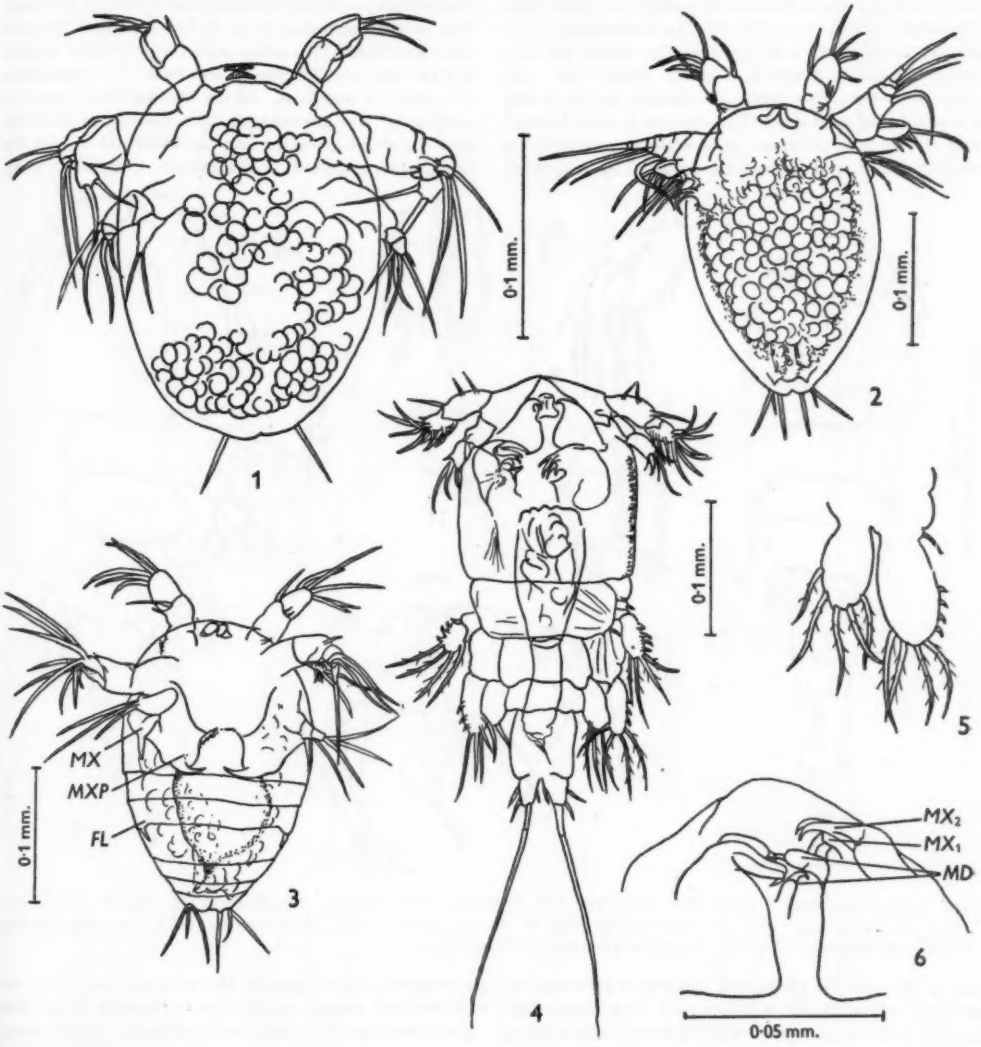
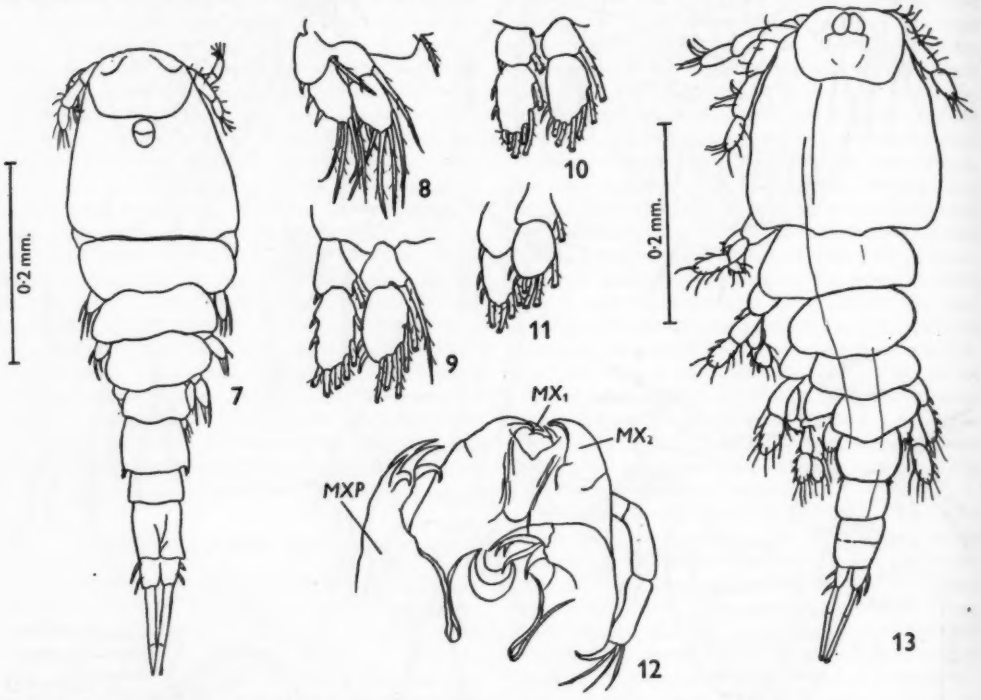


Fig. 1. Nauplius.
Fig. 2. Metanauplius.
Fig. 3. Older metanauplius. *FL*, first leg; *MX*, maxilla; *MXP*, maxillipede.
Fig. 4. First copepodid larva.
Fig. 5. Fourth leg of the first copepodid.
Fig. 6. Mouthparts of first copepodid. *MD*, mandible; MX_1, first maxilla; MX_2, second maxilla.

borne by the body. There are no surface marks. The first antenna is three-jointed. The basal segment is short, while the second is slightly longer than the third in the ratio of 13 : 10. The distal edge of the second joint bears a spine dorsally, while the terminal segment bears a ventral spine and two plumose setae. The biramous second antenna has a two-jointed protopod. The exopod is four-jointed and carries four plumose setae while the endopod is single-jointed and bears two setae. The mandible has a two-jointed protopod, bearing a four-jointed exopod, armed with a spine and four setae, and a single-jointed endopod carrying two plumose setae.

antenna carries two long, and three short, spines on its distal joint, one long and two short spines on the second joint, and a short small spine on the base. The second antenna is as in the nauplius but the endopod bears two setae and three shorter spines, with a very small spine on the base. The mandibles are as in the nauplius. As the larva grows older, the outline of the oesophagus, the distended stomach and the short intestine can be discerned as also the triangular, rounded, anal laminae. Careful focusing

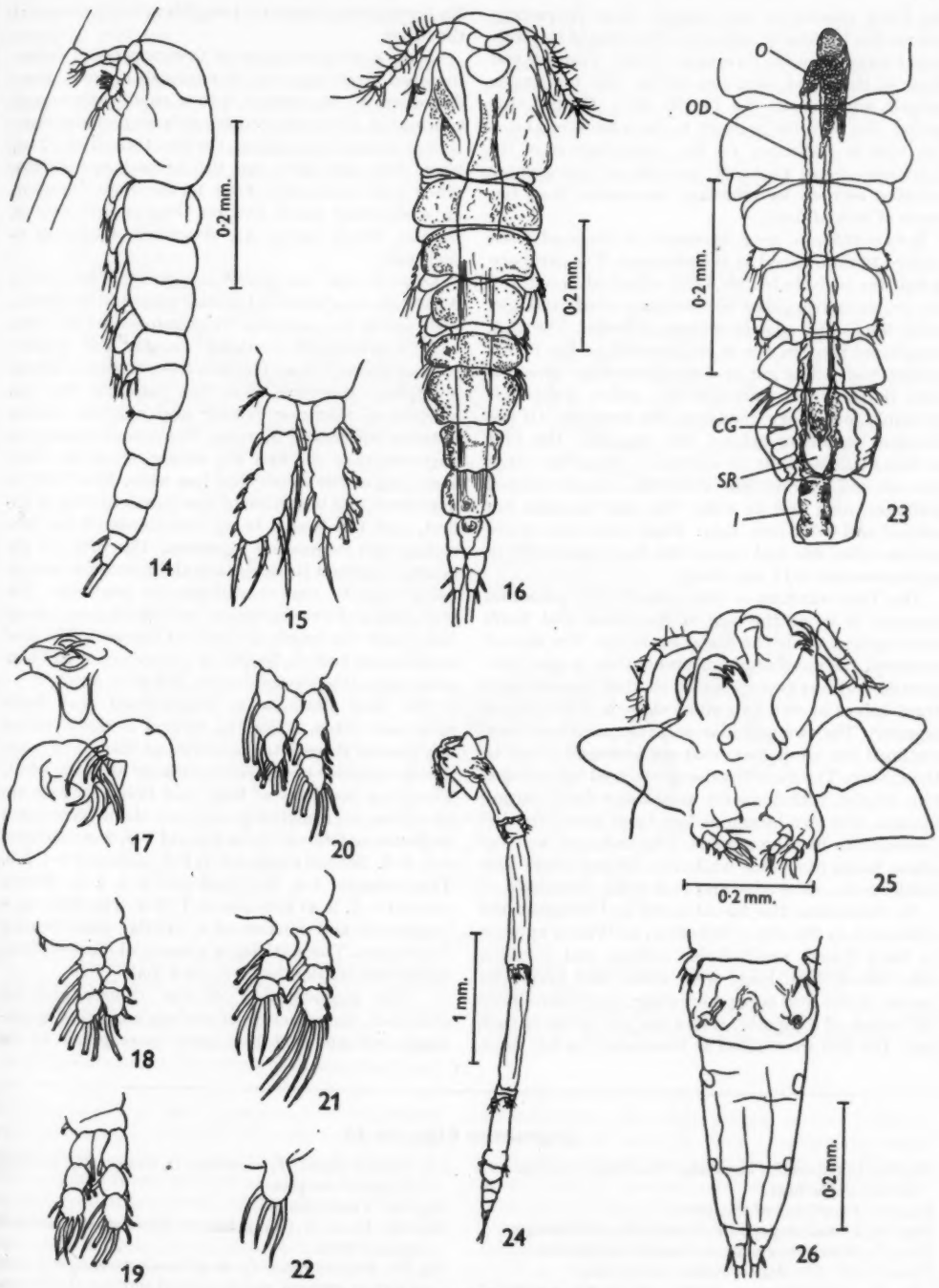


Figs. 7–13. Copepodid 5 days after hatching. Fig. 7. Dorsal view of larva. Fig. 8. First leg. Fig. 9. Second leg. Fig. 10. Third leg. Fig. 11. Fourth leg. Fig. 12. Mouth parts. MX_1, first maxilla; MX_2, second maxilla; *MXP*, maxillipede. Fig. 13. Copepodid 6 days after hatching.

The metanauplius

After swimming about for nearly 24 hr., the nauplius moults into the metanauplius. The body becomes longer and decidedly oval in outline. Exclusive of the anal setae it is 0·220 mm. long and 0·147 mm. broad in the anterior half of the body. The broadly rounded front end is marked by two, pink, median, compound eyes and the acutely rounded narrower posterior end is distinguished by three setae on each side of the body. The first reveals six faint lines on the ventral side. In front of the first groove, spine-like rudiments of the first maxillae can be made out laterally, while more medially the beginnings of the maxillipedes are visible. The third of these grooves indicates the hind-borders of the cephalothorax and shows laterally the incipient first thoracic feet. The succeeding three lines indicate the three thoracic segments and the abdomen.

First copepodid larva. After swimming about for nearly 24 hr., the metanauplius moults into the first copepodid larva, on the third day after hatching. The active and swift movements of these larvae are characterized by a smoothness different from



Figs. 14–26. For legends see p. 152.

the jerky motion of the nauplii. They frequently rest on the bottom or adhere to the sides of the container away from the direction of light. Towards the close of the third day, the larvae rest for longer periods and die on the fourth day. During this period the parasite appears to be infective, and a fish host is necessary for the continuation of its existence—more probably because of the greater aeration secured by effortless movement than because of lack of food.

It is 0·325 mm. long, exclusive of the anal setae and 0·136 mm. broad at the carapace. The carapace is half the body in length, semi-elliptical in outline, the posterior truncated border being nearly straight while the frontal edge is obtusely rounded. The pink compound median eye is conspicuous in the transparent body. The gut is distinguished by greenish yolk in the front part and by yellow lumps of, probably, excretory matter in the intestine. Of the thoracic segments behind the carapace, the first is four-fifths as wide as carapace, while the other two are half as wide. The abdomen is single-jointed 0·05 mm. long and as wide. The anal laminae are conical and 0·025 mm. long. Each bears two spines on the outer side and one on the inner, and ends in a plumose seta 0·18 mm. long.

The first antenna is two-jointed, the proximal segment is twice the size of the distal and bears three spines, while the distal bears ten. The second antenna, the chief organ of prehension, is also two-jointed and has two spines on its first segment and three spines as well as a stout claw on the terminal segment. The two maxillae and the mandibles have assumed the adult form and are arranged closer to the mouth. The maxillipedes are curved appendages two-jointed, with five teeth as well as a claw pointing inward. The two biramous legs have a two-articled protopodite bearing a spine. The endopod, an oval plate, bears five setae while the exopod bears four teeth on the outer edge and four setae terminally.

To determine the larval hosts and to see if the gills serve as the site of infection, as Wilson appears to have found, small *Labeo calbasu* and *Cirrhina reba*, about 2 in. long, were taken and about six larvae of the first copepodid stage, were introduced by means of a pipette, under the gill cover of each fish. The fish were killed at intervals of a few days. No larvae were found on the gills or body of any of the fishes.

Next, a large number of first copepodid larvae (hatched from eggs on 13 September) were introduced on 15 September into a small glass trough containing about six *Oryzias rubristigma* and eight *Barbus stigma*, 2 in. long. On the 18th, 19th, 21st, 23rd, 24th and 26th, one fish of each species was killed and examined. Four larvae were obtained, on 4 different days, two on *Oryzias* and two on *Barbus*. These larvae are described according to their age.

Larva 5 days' old (fourth copepodid). The body is 0·564 mm. long without the anal setae and 0·174 mm. wide across the carapace. The carapace is cuneiform with a truncated posterior margin and slightly convex lateral edges. The first free thoracic segment is slightly narrower than the carapace but has a concave posterior border and slightly convex anterior and lateral margins. The second segment is narrower than the first but longer, while the third is as long as the second but less wide. The fourth is narrowest, the width being one-fourth of that of the first, and the length being two-thirds of the preceding and succeeding segments. The fifth, or the genital, segment is quadrilateral, its anterior margin being slightly narrower than the posterior. The abdomen is of two segments, the front segment being two-thirds the length of the hind-segment. The anal laminae are half the length of the abdomen and bear setae as in the previous stage and as in adult.

The first antenna is four-jointed and bears seventeen setae, while the second is three-jointed and carries three setae and a stout claw. The mandibles, maxillae and maxillipedes are as in the adult. There are six pairs of legs. The first four legs are biramous, the rami being two-jointed and have spines and setae as follows: First exopod 1–1, 2–4; endopod 0–1, 2–5. Second exopod 1–1, 2–6; endopod 0–1, 2–5. Third exopod 1–1, 2–6; endopod 0–1, 2–5. Fourth exopod 0–1, 2–4; endopod 0–1, 2–4. The fifth leg is uniramous and consists of a circular plate bearing four spines. The sixth leg is a vestigial cluster of two spines and marks the larva as a male.

The segmentation of the thorax and the abdomen, the structure of the legs and other appendages indicate that this larva corresponds to the

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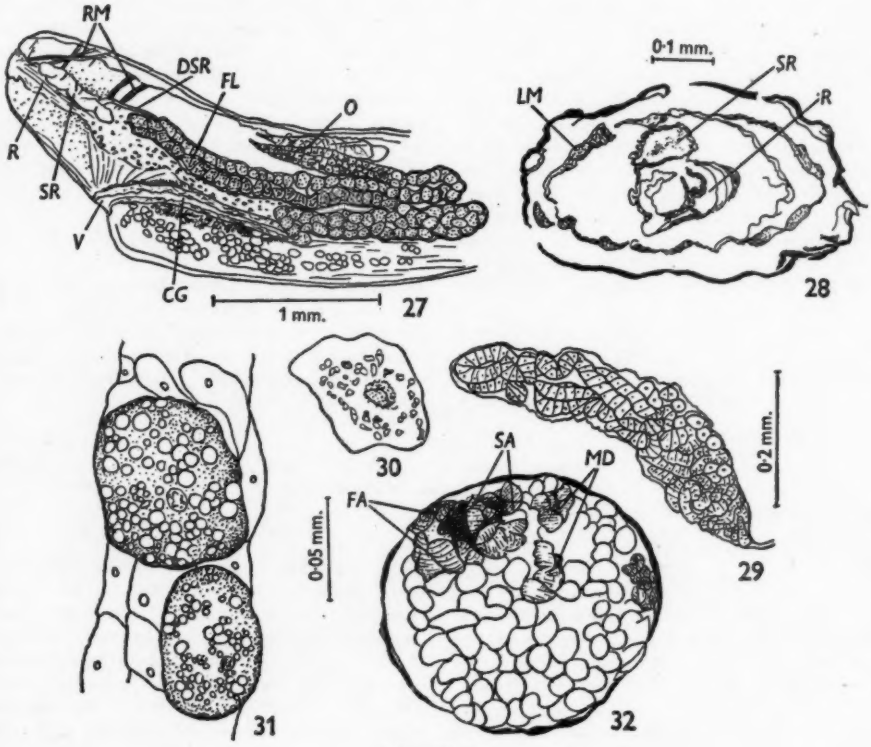
Legends to Figs. 14–26

Fig. 14. Copepodid 8 days after hatching attached to a fin ray of the host.
Fig. 15. Fourth leg of the larva.
Fig. 16. Female copepodid 10 days after hatching.
Fig. 17. Mouth appendages of female copepodid.
Figs. 18–22. Five legs of female copepodid.
Fig. 23. Reproductive organs of female copepodid. *CG*, cement gland; *I*, intestine; *O*, ovary, *OD*, oviduct; *SR*, semen receptacle.
Fig. 24. Young female.
Fig. 25. Head of young female showing rudiments of cephalic arms.
Fig. 26. Posterior end of young female showing reduction of genital segment and backward shifting of fifth legs.

third copepodid described by Wilson and the fourth copepodid of Nakai.

Larva 6 *days' old* (*fourth copepodid*). The body is 0·59 mm. long without the anal setae and 0·2 mm. broad. Besides growth in size the larva shows the posterior segment of the abdomen faintly constricted into two. This three-jointed abdomen distinguishes Wilson's fourth or last copepodid and Nakai's fifth exopod 1–1, 4–5; endopod 0–1, 2–5. The fifth and sixth legs are as in a previous stage.

Larva 8 *days' old* (*fifth copepodid*). This larva corresponds to a stage between Nakai's fourth and fifth copepodids. The body has grown to a length of 0·64 mm., without the anal setae. The abdomen is definitely three-segmented as in adult. The first antenna bears twenty-three spines and setae on its



Figs. 27–32.

Fig. 27. Lateral view of adult. *CG*, cement gland; *DSR*, ducts of semen receptacle. *FL*, forward loop of oviduct; *O*, ovary; *R*, rectum. *RM*, rectal muscles; *SR*, semen receptacle; *V*, vulva.

Fig. 28. Transverse section of abdomen of adult. *LM*, layer of muscles; *R*, rectum; *SR*, semen receptacle.

Figs. 29–31. Developing eggs. Fig. 29. Ovary. Figs. 30 and 31. Ova.

Fig. 32. Embryo showing 'Kopfplatten'. *FA*, first antennae; *MD*, mandibles; *SA*, second antenna.

or final stage. The first antennae are four-jointed and bear twenty-one spines, while the second antennae are three-jointed and bear three marginal spines, three terminal spines and a stout claw. The first four legs have two-articled rami, unlike those of Wilson's fourth and Nakai's fifth copepodids. The spines and setae are as follows: First exopod 1–1, 3–6; endopod 1–1, 2–5. Second exopod 1–1, 4–5; endopod 0–1, 2–5. Third exopod 1–1, 4–5; endopod 0–1, 2–5. Fourth four segments. The three-jointed second antenna carries a stout terminal claw besides four other spines. The mandibles, maxillae and maxillipedes are more closely arranged near the mouth. The rami of the first four legs are two-jointed, with the distal segment slightly divided. The spines and setae are arranged as follows: First exopod 1–1, 4–4; endopod 0–1, 2–5. Second exopod 1–1, 4–5; endopod 0–1, 2–5. Third exopod 1–1, 4–5; endopod 0–1, 2–5.

Fourth exopod 1–1, 4–5; endopod 0–1, 2–5. Fifth and sixth legs as in previous stage.

Larva 10 *days' old* (*sixth copepodid*). This larva, the only female of the four copepodids obtained by artificial infection, measured 0·91 mm. long and 0·20 mm. broad. The carapace is cuneiform as in the male but longer (0·26 mm.) than broad. Because of the proportionately narrow carapace, the body appears longer than in the male, and owing to the fourth and the genital segments being slightly broader than the third, the body appears uniformly thick without the waist-like constriction about the fourth segment noted in the male. The abdomen is of three distinct segments, of decreasing size and the anal laminae have a faint groove across. The first antenna bears twenty-six spines and setae as in adult, while the second antenna similarly carries six. The first four legs are biramous, the rami being three-jointed as in adult. The spines and setae are arranged as follows: First exopod 1–1, 3–5; endopod 0–1, 0–2; 2–4. Second exopod 1–1, 1–1, 2–5; endopod 0–1, 0–2, 2–4. Third exopod 1–1, 1–1, 3–5; endopod 0–1, 0–2, 2–4. Fourth exopod 1–1, 1–1, 3–5; endopod 0–1, 0–2, 2–4. This corresponds to the final stages described by Wilson and Nakai, as the fourth and fifth copepodids respectively.

Table 1

	Cephalothorax	First thoracic segment	Second segment	Third segment	Fourth segment	Genital segment	Abdomen and anal laminae	Anal setae
Mature copepodid	53	14	18	17	10	17	53	70
Young female	60	110	225	210	100	17·5	70	75

Young female

The two gourami brought alive bore not only adult egg-bearing parasites, but also a few ulcers on the body and fins. These areas were scraped and two young females were obtained from under the scales. The body of each female was long, slender and cylindrical. Exclusive of anal setae this form measured 5·93 mm. long and 0·22 mm. broad across the thoracic legs and 0·17 mm. across the rest of the body. The cephalothorax is 0·3 mm. long and 0·23 mm. broad at the posterior margin and measures 0·505 mm. across the tips of the budding cephalic arms. Each arm is a triangular foliaceous extension of the posterior region of the cephalothorax. The first four segments are 0·505, 1·12, 1·05 and 0·5 mm. in length, while the genital segment and abdomen are 0·087 and 0·35 mm. long and the anal setae measure 0·325 mm. The proportionate elongation of the different parts of the body of the young female as compared with those of the mature larva can be indicated as shown in Table 1.

It will be obvious that the cephalothorax, the genital segment, the abdomen and the anal setae have changed but little, whereas the four thoracic segments have lengthened 8, 12, 12, 10 times in the order mentioned. Changes in the disposition of the reproductive organs also are of interest. In the larva the ovary is located in the first free thoracic segment and the segment fused with the cephalothorax, whereas in the adult it is found in the fourth thoracic segment. The oviduct has lengthened far out of proportion to the growth of the body, and is thrown into a loop and a half and has three limbs. The semen receptacle lying in the genital segment of the larvae appears pushed back into the abdomen in the adult. The cement glands are conspicuous in the lateral region of the genital segment with the oviducts appearing to twist through them. In the adult, however, there are no twists in this final section of the oviduct which becomes drawn out into a long straight tube (called cement gland by Wilson) with glandular walls.

DISCUSSION

In his account of the genus *Lernaea*, Wilson (1917*b*) says 'there is no semen receptacle connected with the oviduct, at least none is revealed either in gross examination or in sections and hence it is impossible to prove just where the egg fertilized' (p. 181). It is likely that Wilson did not examine sections of the body posterior to the vulvae, since the semen receptacle though thin-walled and median as in the Ergasilids is unmistakable. In gross examinations it can be located by following its stout ducts which open into the oviducts where these loop forwards. Though the details of fertilization can be followed only in suitably preserved material, it is probable that the eggs are fertilized in the oviducts, beyond the opening of the ducts.

The blastodermic cuticle, regarding the absence of which Wilson is not definite, was not found in any of the sections of the developing eggs. The formation of this cuticle, considered by Korschelt and Heider as an ecdysis of the suppressed nauplius, is hardly to be expected in this genus *Lernaea* in which the egg hatches into a nauplius and the development is continuous and graded. Though in the present species there are only two well-marked naupliar stages, the nauplius and the metanauplius described above, yet close study of a large number of these larvae, reveals four definite grades—the newly hatched nauplius, elliptical in outline differing from the older more oval nauplii and the young metanauplius different from the older metanauplii with

distinct segmentation, rudiments of maxillae, maxillipedes and the first legs. Even in the copepod phase, the development is gradual and continuous. Wilson described four copepodids stage, while Nakai found that in *L. elegans* another stage occurs between the second and third copepodids of Wilson. In the present form a fresh stage was found preceding the final stages of these authors. Though such an addition will not make the total for the genus exceed the six stages found in free-living copepods, yet it must be remembered that some of the distinctions between the stages so far recognized may prove to be minor changes accompanying each moult. Further, till a knowledge of the development of a number of species helps us to interrelate the stages occurring in any one with those found in others the enumeration of the copepodid stages must remain arbitrary and tentative.

The duration of each copepodid stage and the time taken for the appearance of the sexually mature larva may vary with different species. Wilson states that the first copepodid larva appears on the third day and that 'each copepodid stage occupies from 10 to 14 days'. Nakai's observations, however, have not been uniform and consistent. His nauplii and metanauplii lived 3 and 4 days or died earlier, and the first copepodids survived for 6 days and even changed into the next stage. In the present form the nauplius lives for 24 hr., as the metanauplius also does, and the first copepodid appears on the third day, as Wilson found. But the first copepodids always lived only for 24 hr. and died thereafter. If, however, suitable fish hosts were available, as in the experiment, they reached the final mature copepodid stage 10 days after hatching. This rapid progress through all the larval stages, as well as the incidence of sex characters even on the fifth day, are probably features peculiar to this tropical form.

The nature and number of hosts needed to complete the life cycle may not be the same for all species of the genus. Wilson observes that 'if the larvae thus far discovered can be taken as typical of the entire genus, every species of *Lernaea* has at least two hosts'. Nakai's finding the copepodid stages of *L. elegans* on *Cyprinus carpio*, one of the many hosts of the adult, shows that a temporary or intermediate host is not absolutely necessary for the completion of the life cycle. As the adult of the present species, *Lernaea chackoensis*, is parasitic on widely different hosts such as *Osphronemus goramy*, *Catla catla*, *Gobius griseus* and *Oryzias melastigma*, it is possible that the larvae also are not handicapped by any specific limitation in the choice of their hosts. In the present study both the small fish (*Barbus stigma* and *Oryzias rubristigma*), experimentally exposed to infection, were parasitized. Further experiments on the infection of smaller young of the adult hosts, may show that while the first copepodids, tiny as they are, may obtain a ready hold on small-sized fish, the rapidly growing young female may not be able to retain its position except on certain parts of the smaller fish, and may perish or may succeed to attach themselves to fish they failed to infect as larvae. Thus, while the parasite may complete the life cycle on one host alone, it appears capable of changing its location or its host till it metamorphoses into the adult.

Wilson (1914) has discussed the mutual antagonism between the copepodid larvae of *Lernaea* and mussel glochidia and methods of controlling the lernaeids. As there is no other parasite found on the gourami or catla, only the exclusion from the ponds, of smaller fish which may offer suitable attachment to these minute larvae, may check the parasitization of these important culture fish. Since the parasites become very scarce after the first monsoon showers when the ponds overflow, it is likely that frequent draining of water from the culture tanks may remove the larvae.

SUMMARY

1. The nauplius, the metanauplius and the first copepodid larva, of *Lernaea chackoensis* were reared *in vitro* and are described.

2. The four copepodid larvae obtained by artificial infection of *Barbus stigma* and *Oryzias rubristigma* are described.

3. The young females infesting the adult host fishes are described, noting the changes which take place after the copulation of the female larva.

4. The accelerated development of this tropical form and the need for a temporary host to complete the life cycle are discussed.

The author thanks Mr G. Gopalakrishna Nair, M.A., of the Department for sketching some of the larvae and assisting in the experiments.

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THE EFFECT OF THE PARASITIC COPEPOD, *MYTILICOLA INTESTINALIS* (STEUER) UPON THE CONDITION OF MUSSELS

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(With 3 Figures in the Text)

INTRODUCTION

Mytilicola intestinalis (Steuer) was first recorded in Britain by Ellenby (1947) in mussels from Blyth, Northumberland, but Dr J. P. Harding informs us that a single specimen was sent to the British Museum from Portsmouth by Miss L. Beanland in December 1937. It had previously been recorded from the Mediterranean (Steuer, 1902, 1903 and Monod & Dollfus, 1932) and from the German North Sea coast in the Bremerhaven-Cuxhaven area (Caspers, 1939).

Since Ellenby's original British discovery we have received personal communications from J. N. R. Grainger (Trinity College, Dublin) and A. R. Hockley (University College, Southampton) reporting the discovery of the parasite in Cork Harbour, Eire, and at several points on the south and south-west coasts of England.

We have examined samples of mussels from Conway and Montrose, Scotland, without finding any parasites, but few other localities on the east or west coast of Britain have so far been examined. A detailed survey of the mussel beds of commercial importance is being planned.

A cursory examination of mussels received from Blyth showed that the majority were in very poor condition. In view of the importance of mussels as food and as bait it was deemed advisable to examine the effect of the parasites upon the condition of the host.

METHODS

A large sample of mussels from Blyth was obtained in January 1948 from the Dove Marine Laboratory, Cullercoats. On arrival the mussels were carefully measured and grouped in 0·5 cm. size groups. The mussels were opened without damage to the gut and the meats were removed entire from the shell and placed in sea-water Bouin fixative, being subsequently transferred to 70% alcohol. The individual mussels in each size group were weighed from alcohol after careful blotting.

Mussels from the most numerous size group (5·5–5·9 cm.) were dissected individually, under a magnification of approximately × 3, in small dishes. The gut was opened along its entire length. All parasites seen were removed and when the dissection was complete, the teased-out tissues and fluid were systematically searched on a squared counting plate under a binocular microscope. All parasites were measured to the nearest quarter millimetre. The dissection and the subsequent examination of teased-out tissues and liquid were so thorough that the number of parasites missed must have been negligible.

No parasites were found in the oesophagus, stomach or style sac, but parasites in all stages were numerous throughout the remainder of the gut.

No attempt was made in this preliminary study to sex the parasites but individuals with external egg sacs were noted.

OBSERVATIONS AND DISCUSSION

Tables 1–6 give particulars of the numbers of large (over 1·5 mm.) and small parasites found in fifty mussels received from Blyth of shell length 5·5–5·9 cm. inclusive. This size group has been further subdivided according to the weight of flesh into 0·5 g. subgroups. Each subgroup is tabled separately.

Table 1. *Showing numbers of large and small* Mytilicola *found in mussels of shell length* 5·5–5·9 *cm. and flesh weight* 2·0–2·45 g.

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No. of large *Mytilicola* over 1·5 mm.
2·0	17	1	16
2·05	28	14	14
2·05	14	8	6
2·25	41	25	16
2·35	11	0	11
2·45	6	0	6
2·45	32	5	27
2·45	23	9	14
Mean 2·26	21·50	7·75	13·75

It will be seen that both the mean number of parasites per mussel and the mean number of large parasites varies little in mussels of flesh weight 2·0–3·45 g., but that above this weight there is a steady decline in the degree of parasitism (Fig. 1).

Mussels which have reached a flesh weight of more than 3·45 g. at shell length 5·5–5·9 cm. show, generally speaking, a lower intensity of infection with parasites than mussels in the lighter subgroups. Expressed in another way—above a flesh weight of 3·45 g. the condition of the mussels is inversely related to their content of parasites. This inverse relationship is shown in Fig. 2.

Table 2. *Showing numbers of large and small* Mytilicola *found in mussels of shell length 5·5–5·9 cm. and flesh weight 2·5–2·95 g.*

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No of large *Mytilicola* over 1·5 mm.
2·60	21	7	14
2·60	59	34	25
2·65	30	10	20
2·65	46	14	32
2·70	5	1	4
2·75	10	4	6
2·75	28	17	11
2·75	29	25	4
2·80	31	0	31
2·85	16	6	10
2·85	15	3	12
2·95	5	0	5
Mean 2·74	24·58	10·08	14·50

Table 3. *Showing numbers of large and small* Mytilicola *found in mussels of shell length 5·5–5·9 cm. and flesh weight 3·0–3·45 g.*

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No of large *Mytilicola* over 1·5 mm.
3·00	13	9	4
3·00	25	11	14
3·00	45	32	13
3·10	55	45	10
3·15	5	0	5
3·20	10	1	9
3·25	18	0	18
3·30	13	1	12
3·35	12	0	12
3·45	6	2	4
Mean 3·18	20·20	10·10	10·10

The sample of mussels from Blyth contained none without parasites. In order to compare the flesh weight of parasitized and unparasitized mussels, a sample of the same shell length (5·5–5·9 cm.) was collected from the Conway estuary. This second sample was collected in July when mussels are generally in a slightly poorer condition than during January, since in the latter month the gonads are practically mature. Table 7 gives a comparison of the weight of meats in the two samples. The Conway mussels are strikingly superior, and although this estuary has the reputation of producing fatter mussels than many other beds, one is led to the conclusion that the exceptionally poor condition of the Blyth mussels was the result of heavy infestation by *Mytilicola*.

Table 4. *Showing numbers of large and small* Mytilicola *found in mussels of shell length 5·5–5·9 cm. and flesh weight 3·5–3·95 g.*

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No. of large *Mytilicola* over 1·5 mm.
3·50	4	0	4
3·50	15	2	13
3·50	21	1	20
3·50	26	17	9
3·55	7	1	6
3·55	11	3	8
3·55	15	0	15
3·60	9	0	9
3·65	10	0	10
3·65	28	18	10
3·70	6	1	5
3·95	2	1	1
3·95	13	0	13
Mean 3·63	12·85	3·40	9·45

Table 5. *Showing numbers of large and small* Mytilicola *found in mussels of shell length 5·5–5·9 cm. and flesh weight 4·0–4·45 g.*

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No. of large *Mytilicola* over 1·5 mm.
4·00	16	6	10
4·05	15	1	14
4·05	8	0	8
4·15	6	0	6
Mean 4·06	11·25	1·75	9·50

Table 6. *Showing numbers of large and small* Mytilicola *found in mussels of shell length 5·5–5·9 cm. and flesh weight 4·5–4·95 g.*

Flesh weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No. of large *Mytilicola* over 1·5 mm.
4·65	7	6	1
4·70	5	0	5
4·80	5	0	5
Mean 4·72	5·7	2·0	3·7

A possible alternative conclusion from the data presented would be that the variation in the number of parasites per mussel was the consequence of initial differences in their condition and vitality, stronger mussels being able in some way to resist

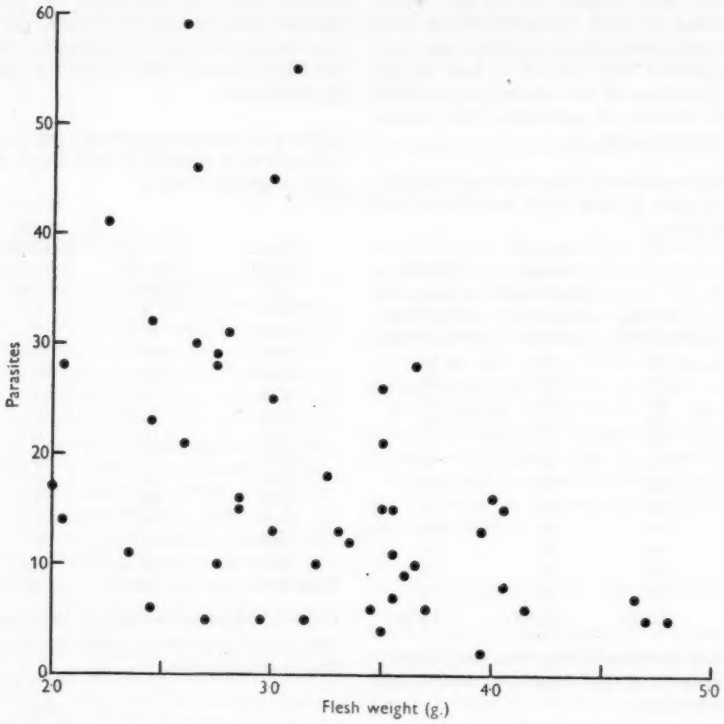

Fig. 1. Total number of parasites per mussel plotted against weight of flesh.

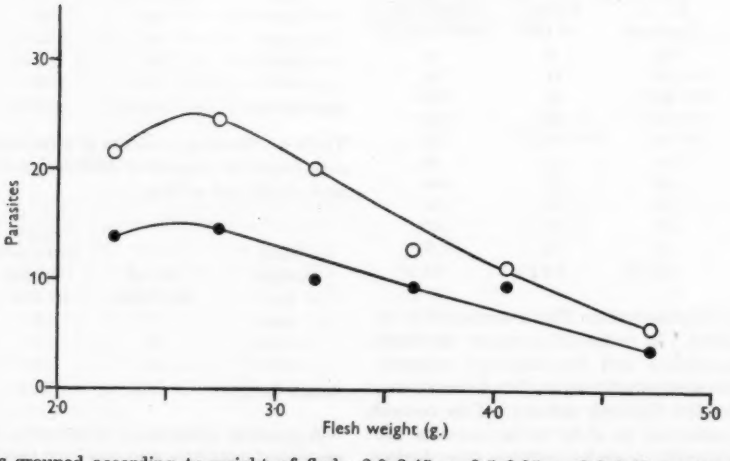

Fig. 2. Mussels grouped according to weight of flesh; 2·0–2·45 g., 2·5–2·95 g., 3·0–3·45 g., etc. Mean number of parasites (all sizes—open circles) and mean number of parasites over 1·5 mm. (solid dots) per mussel, in each group, plotted against mean flesh weight.

infestation by parasites, while weaker mussels succumbed. The information available does not permit us to separate cause and effect with any certainty, but after much experience in the examination of mussels from different parts of our coasts we consider that the heavily parasitized mussels from Blyth were more emaciated than is commonly found in the normal range of variation. It is possible that further investigations may reveal a geographical pattern, with poor condition and high count of parasites, associated with poor environmental conditions.

Table 7. *A comparison of flesh weights of mussels of shell length 5·5–5·9 cm. from Blyth (parasitized) and Conway (not parasitized).*

Locality	No.	Mean flesh weight per mussel (g.)	Range (g.)
Blyth	50	3·206	2·0 –4·8
Conway	52	5·975	4·35–7·70

In addition to the size group 5·5–5·9 cm., the two largest mussels (7·0–7·4 cm.) in the sample and the eight smallest (3·5–4·4 cm.) were also dissected; particulars of the parasites found in them are given in Table 8. Some of the very small mussels were so heavily infested, relative to their size, that it is difficult to imagine their survival. The low intensity of infection in the large mussels suggests that they were able to reach this size and weight by virtue of their small content of parasites. Little is known, however, as yet regarding the duration of life in *Mytilicola*, or the seasonal variation in numbers of parasites, although these matters are under investigation.

Caspers (1939) stated that Haranghi, working at Heligoland, observed poor development of the style in infected mussels. Haranghi also found that infected mussels needed 12 hr. or more to filter off a suspension of indian ink from 1 l. of water, whereas normal mussels cleared this volume in 7 hr. So far as we are aware no further account of these experiments has been published.

Odlaug (1946) has investigated the effect of *M. orientalis* (Mori) on the condition of the Olympia oyster (*Ostrea lurida*) and, although the results are masked to some extent by the effect of spawning upon the condition of the oysters, he concluded that the parasites adversely affected condition.

In this investigation several thousand oysters were dealt with in a short time and, although the number of parasites per oyster was usually small, the results suggest that some at least of the smaller parasites were overlooked. This would, however, be unlikely to affect the validity of the conclusions reached . After dissecting fifty mussels the writers, working as a team, were still unable to complete a dissection and the measurement of the parasites (which in itself took no more than a few minutes) in substantially less than half an hour. In illustration of the difficulty of detecting all parasites, particularly in fresh material, it is relevant to recall that Ellenby (1947), examining the same material from Blyth as that analysed herein, found a maximum infestation of only eighteen per mussel. Of the sixty mussels examined in this investigation no less than nineteen contained a larger number of parasites. The stages below 1·5 mm. are very easily missed unless the teased-out material is systematically searched under a binocular microscope.

Table 8. *Showing the number of* Mytilicola *in small and large mussels from Blyth*

	Size group (cm.)	Flesh-weight (g.)	No. of *Mytilicola*	No. of small *Mytilicola* 1·5 mm. or less	No. of large *Mytilicola* over 1·5 mm.
Small mussels	3·5–3·99	0·40	5	—	5
		0·50	13	1	12
	4·0–4·49	0·91	4	—	4
		0·92	54	50	4
		0·98	7	1	6
		1·17	4	1	3
		1·37	3	—	3
		1·85	24	17	7
Large mussels	7·0–7·49	4·3	11	3	8
		5·5	14	10	4

The length distribution of all *Mytilicola* found in Blyth mussels of shell length 5·5–5·9 cm. is shown in Fig. 3. The measurements of adult females do not include external egg-sacs. For the purpose of this figure the parasites have been placed in 1 mm. size groups (0·0–0·95, 1·0–1·95, etc.). The smallest parasite seen measured 0·3 mm. and corresponded to the stage at which infection of mussels is stated to occur (Caspers, 1939). The largest *Mytilicola* found measured 9·0 mm., but Ellenby (1947) records parasites over 10 mm. in length, but does not say whether this includes the egg-sacs; presumably his specimens were measured living, whereas ours were fixed and possibly slightly contracted. A high proportion of the females 7·0 mm. or over in length showed external egg-sacs.

It is probable that the three peaks in the curve represent juveniles, males and females respectively, although the second peak may include a proportion of immature females. Steuer (1902) gives the length of adult males as approx. 4 mm. and of adult females as approx. 8 mm.

From the large number of juvenile *Mytilicola* present in Blyth mussels in January, and the substantial proportion of females with egg-sacs, it appears that breeding occurs throughout the winter. The adult males and females found in January were therefore at least 1-year old.

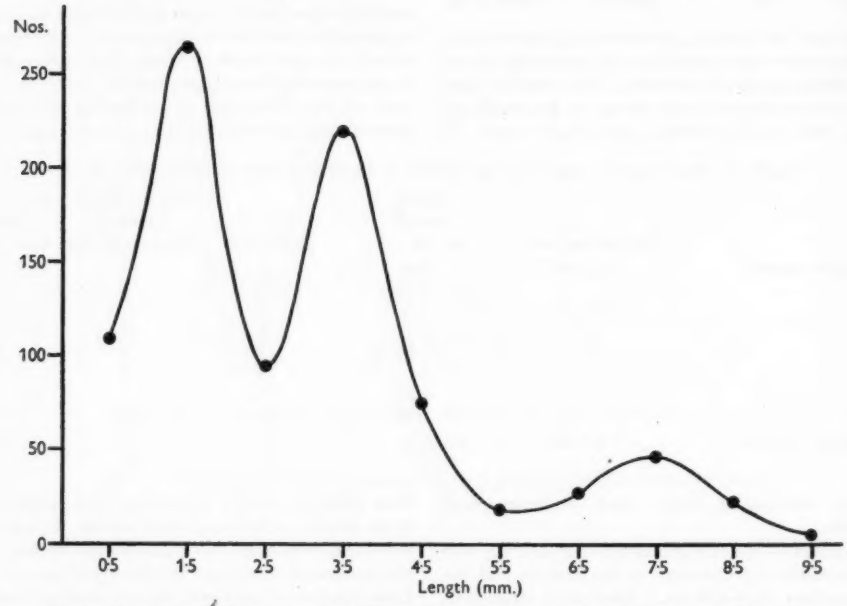

Fig. 3. Frequency distribution of *Mytilicola* from Blyth mussels 5·5–5·9 cm. shell length.

In addition to being found at Blyth, *Mytilicola* is widespread, though possibly scattered, on the south coast from Kent to Devon (private communication from A. R. Hockley). It also occurs at Cork (J. N. R. Grainger), and probably in several other localities. Its known distributions suggests transport in mussels attached to vessels plying between the several ports at which it has been found. It is likely, therefore, that the parasite will become more widely dispersed. At present it has not been found on any bed of mussels of commercial importance and, until more is known of the ecology of the parasite, the conditions in which it might be expected to become abundant cannot be stated. Few of the important commercial beds have so far been examined, but steps have been taken to start such an investigation immediately. Further information on the life history and ecology of the parasite may be expected from the researches of Grainger and Hockley.

CONCLUSIONS AND SUMMARY

1. *Mytilicola intestinalis* (Steuer) was found in 100% of mussels examined from Blyth, Northumberland. These mussels varied in length between 3·5 and 7·5 cm.

2. The degree of infestation varied from two to fifty-nine parasites per mussel.

3. There was an inverse relationship between the condition of mussels of the size group 5·5–5·9 cm. and both the mean number of parasites per mussel and the mean number of parasites over 1·5 mm. in length per mussel.

4. The mean weight of flesh per mussel in the size group 5·5–5·9 cm. (shell length) was 3·206 g. from Blyth (parasitized) and 5·975 g. from Conway (not parasitized).

5. It is concluded that the presence of *Mytilicola* is associated with a serious reduction of condition in infested mussels.

We are indebted to J. N. R. Grainger of Trinity College, Dublin, A. R. Hockley of University College, Southampton, and Dr J. P. Harding of the British Museum for information and material very kindly placed at our disposal prior to publication.

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VALLISIOPSIS CONTORTA N.G. AND N.SP. AND *GASTROCOTYLE INDICA* N.SP., MONOGENETIC TREMATODES FROM MARINE FISHES OF THE MADRAS COAST

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(With 11 Figures in the Text)

INTRODUCTION

Two new monogenetic trematodes of the sub-order Polyopisthocotylea Odhner were obtained from the gills of marine fishes. The characters of the first (*Vallisiopsis contorta*) agree with those of the sub-family Vallisiinae Price (family Discocotylidae Price) except in the presence of accessory sclerites in the clamp skeleton. As the mere presence of accessory sclerites does not justify the creation of a new sub-family, I refer this species to the sub-family Vallisiinae the diagnosis of which should, however, include the presence of accessory sclerites as an additional character. The second species described belongs to the sub-family Gastrocotylinae Sproston (family Microcotylidae Taschenberg).

1. **Vallisiopsis** n.g.

Vallisiinae with both the body and the haptor asymmetrical; gonads lying in the posterior half of the body; testes follicular, overlapping the anterior part of the ovary and also extending anterior to it; genital pore armed with hooks; cirrus armed; oesophagus long, extending below the genital pore; haptor with four clamps on either side and one pair of anchors; clamp skeleton discocotylid in type with one pair of accessory sclerites; eggs spindle-shaped with polar filaments.

Vallisiopsis contorta n.g. and n.sp.

With the characters of the genus; genital corona consisting of twenty or twenty-one hooks; vaginal pore lateral below the level of the common genital pore; ovary in the form of a double loop.

Description. Fifty-six specimens of *Lactarius lactarius* (Bl. Schn.) were examined on various dates. Of these fourteen fish yielded *Vallisiopsis contorta*. In all sixty-five parasites were collected. The parasites were found between the two rows of gill filaments and were firmly attached to them with the aid of the clamps. The maximum length of the parasites was 3·5 mm.

These parasites show marked asymmetry. The body is bent on itself and also twisted. In the normal position the anterior part of the body lies at right angles to the posterior part, and the dorso-ventral axis of the anterior part is twisted through about 90° (Fig. 2). Just at the region where the body is flexed there is a flap-like outgrowth.

The entire surface of the body is transversely ringed, in consequence the sides have a serrated appearance, which in certain regions is more pronounced than at others (Fig. 1*a*, *b*). The posterior end of the body bears a pair of anchors, which are 23 μ long. The haptor is asymmetrical. There are four clamps on each side of the haptor. Those on one side lie close together and are nearer the anchors, while those on the opposite side are farther apart and are borne on distinct lobes of the body. The clamp skeleton is typically discocotylid in pattern and bears a pair of accessory sclerites in connexion with the middle piece, which are very similar to the 'V-shaped' pair of sclerites present in Gastrocotylinae. The two rami of the middle piece are joined in some, while in others they remain quite distinct. The width of the clamp is 58 μ.

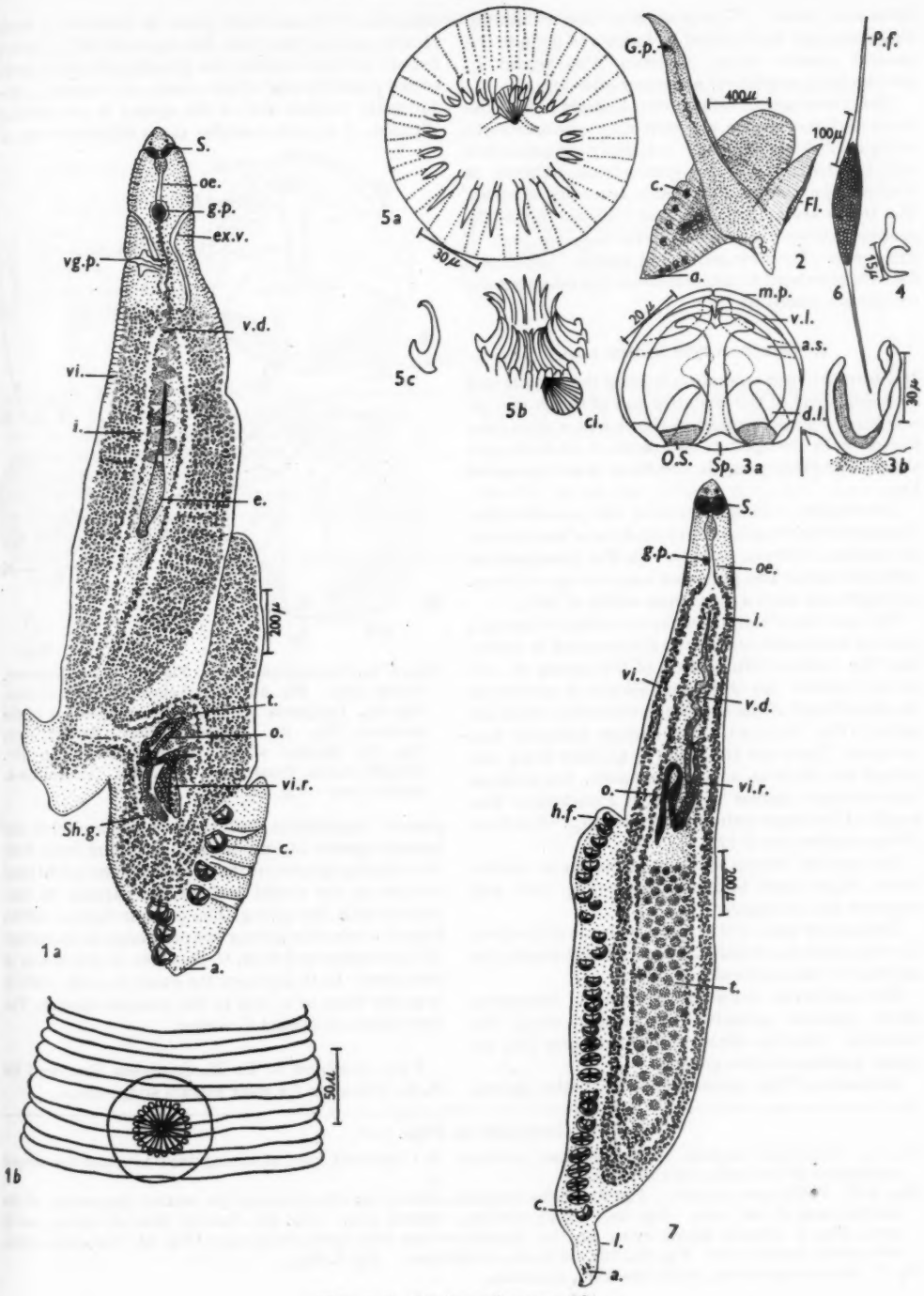
The oesophagus is long and extends below the genital pore. The intestinal crura are branched. The two limbs of the intestine join together posterior to the gonads. Black pigment granules are present all along the intestine.

The testes are follicular, overlap the anterior half of the ovary and extend beyond it. The ovary is in the form of a double loop extending posterior to the testes and is crossed by the transverse vitelline duct. The vitelline reservoir lies adjacent to the ovary. The vitellaria are follicular and coextensive with the intestinal rami.

The parasites when placed overnight in sea water laid a large number of eggs. The eggs are spindle-shaped and have polar filaments. Dimensions are: length, 750 μ, including polar filaments; 200 μ, excluding polar filaments; width, 50 μ.

In one specimen mounted in balsam, the egg in the uterus was peculiar, in that the posterior end did not possess a polar filament, but was rounded and knob-like. It is probable that the polar filament might have been damaged by some accident and regeneration might have occurred.

Discussion. The characters of the present genus agree with those of the subfamily Vallisiinae mentioned by Sproston (1946), except in the presence of accessory sclerites. I do not consider it justifiable to separate this genus from Vallisiinae, on this



Figs. 1–7. For legends see p. 164.

difference alone. I propose that the subfamily diagnosis may be modified as follows to include the present genus: clamp skeleton discocotylid in pattern with or without accessory sclerites.

The present genus differs from *Vallisia* in that the body and the haptor are strikingly asymmetrical, while in *Vallisia* the body is slightly asymmetrical and the haptor is quite symmetrical. Unlike in *Vallisia* the clamps here have accessory sclerites. The testes are all pre-ovarial in *Vallisia*, but in the present species they overlap the anterior part of the ovary. The new genus and species *Vallisiopsis contorta* is created to accommodate the monogenetic trematode described.

2. **Gastrocotyle indica** n.sp.

Haptoral frill not extending beyond the gonads and stopping short of the posterior end of the ovary; an accessory sclerite present in the dorsal wall in connexion with the spring; two pairs of anchors on a short languette; ovary in the form of an elongated loop.

Description. 755 specimens of this parasite were obtained from the gills of fifty-four out of seventy-six specimens of *Caranx kalla* Cuv. & Val. examined on different dates. The parasites measure up to 3 mm. in length and have a maximum width of 530 μ.

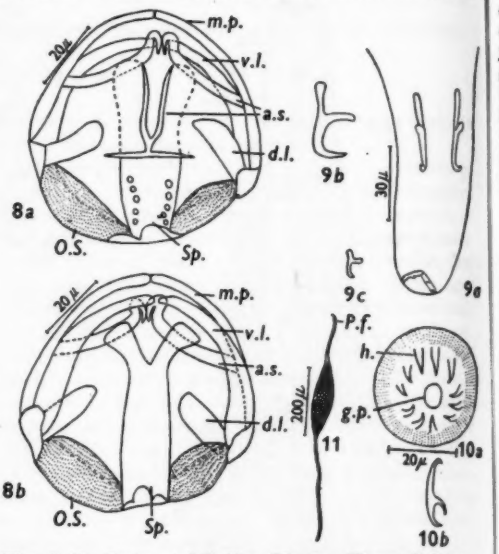
The number of clamps varies according to the age, and the maximum number so far recorded is thirty-six. The ventral bifurcations of the spring do not divide further. An additional sclerite is present in the dorsal wall of the capsule in connexion with the spring (Fig. 8*b*, *a.s.*). The clamps measure 42 μ in width. There are two pairs of anchors lying one behind the other on a short languette. The anchors have a shape similar to those of *Pseudaxine*. The length of the larger pair of anchors is 35 μ, while that of the smaller pair is 12 μ.

The genital corona consists of eleven or twelve hooks which have bifurcate bases (Fig. 10*b*) and measure 19 μ in length.

The greater part of the ovary, which is in the form of a loop with the distal end directed backwards, lies anterior to the haptoral frill (Fig. 7, *o.*).

The intestinal crura are branched. Numerous black pigment granules are present along the intestine. The two limbs of the intestine join together posterior to the gonads.

Discussion. This species agrees with the generic diagnosis of *Gastrocotyle* given in Sproston's work (1946), except that, first, the haptoral frill does not extend forward beyond the gonads and stops short of the posterior end of the ovary, and secondly, the bifurcate ventral end of the spring is not further divided. I do not consider these differences as of



Figs. 8–11. *Gastrocotyle indica.* Fig. 8*a*. Clamp skeleton, dorsal view. Fig. 8*b*. Clamp skeleton, ventral view. Fig. 9*a*. Languette showing the arrangement of the anchors. Fig. 9*b*. Larger anchor, lateral view. Fig. 9*c*. Smaller anchor, lateral view. Fig. 10*a*. Genital corona, front view. Fig. 10*b*. Genital hook, lateral view. Fig. 11. Egg.

generic importance and hence have included the present species in *Gastrocotyle*. It differs from both the existing species in the presence of an additional sclerite in the dorsal wall of the capsule in connexion with the spring. The present species differs from *G. trachuri* in having two pairs of anchors instead of three pairs and from *G. japonica* in the shape of the ovary. In *G. japonica* the ovary is oval, while it is in the form of a loop in the present species. The new species is named *G. indica*.

I am indebted to Dr H. Srinivasa Rao and Dr N. K. Panikkar for their helpful suggestions.

Legends to Figs. 1–7.

Fig. 1*a*. *Vallisiopsis contorta*, entire specimen, mounted. *b*. *V. contorta*, part of *a* magnified to show the ringed appearance of the surface of the body.

Figs. 2–6. *Vallisiopsis contorta*. Fig. 2. Entire specimen drawn from life showing the natural disposition of the various parts of the body. Fig. 3*a*. Clamp skeleton, ventral view. Fig. 3*b*. Lateral view of clamp, partly open. Fig. 4. Anchor, lateral view. Fig. 5*a*. Genital corona with cirrus, front view. Fig. 5*b*. Genital corona, with cirrus, lateral view. Fig. 5*c*. Genital hook, lateral view. Fig. 6. Egg.

Fig. 7. *Gastrocotyle indica*, entire specimen, mounted.

Key to lettering

a.	anchor.	*o.*	ovary.
a.s.	accessory sclerite.	*oe.*	œsophagus.
c.	clamp.	*o.s.*	optical section of anterior wall of the capsule.
ci.	cirrus.	*p.f.*	polar filament.
d.l.	dorsal loop.	*s.*	sucker.
e.	egg.	*sh.g.*	shell gland.
ex.v.	excretory vessel.	*sp.*	spring.
fl.	flap.	*t.*	testis.
g.p.	genital pore.	*v.d.*	vas deferens.
h.	hook.	*vg.p.*	vaginal pore.
h.f.	haptoral frill.	*vi.*	vitellaria.
i.	intestine.	*vi.r.*	vitelline reservoir.
l.	languette.	*v.l.*	ventral loop.
m.p.	middle piece.		

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STUDIES ON THE LUNG NEMATODES OF SHEEP AND GOATS IN THE LEVANT

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(With Plate III and 48 Figures in the Text)

Sheep breeding is an ancient branch of agriculture in the Levant, and its success or failure in this area, as in other countries, intimately depends on the knowledge of parasitic diseases. Comparatively little investigation of this problem has, however, hitherto been undertaken and lung nematodes, which at times constitute a menace to farm management, have not been studied in this country at all. The present study aims at determining the species of lungworms occurring in this geographic area and at elucidating their life histories. Experimental material was collected in local slaughterhouses. Although the origin of each of the hosts could not be ascertained, it was known that during the period investigated sheep and goats were brought to the Jerusalem market not only from local farms, but also from Lebanon, Syria, Transjordan and Turkey. Since sheep-raising conditions are rather similar in all of these countries, it may safely be assumed that the results reflect conditions in them all.

The following species of lungworms were found:

(1) *Dictyocaulus filaria* (Rudolphi, 1809) Raillet & Henry, 1907.
(2) *Cystocaulus ocreatus* (Raillet & Henry, 1907) Micaĉiĉ, 1939.
(3) *Muellerius capillaris* (Mueller, 1889) Cameron, 1927.
(4) *Protostrongylus rufescens* (Leuckart, 1865) Kamensky, 1905.
(5) *Neostrongylus linearis* (Marotel, 1913) Gebauer, 1932.†

Although these species have been known for decades, details of their morphology and life histories have been made available only recently. In spite of numerous studies devoted to this subject, many particulars concerning anatomy and development are still unknown or available information is conflicting. New detailed description of these species have therefore been given and experimentally produced life histories have been described.

* This study was carried out in the Department of Parasitology, Hebrew University, The author wishes to acknowledge his appreciation to Prof. G. G. Witenberg for helpful advice and criticism.

† *N. linearis* has been found only twice. The material was, however, insufficient for detailed study or experiments.

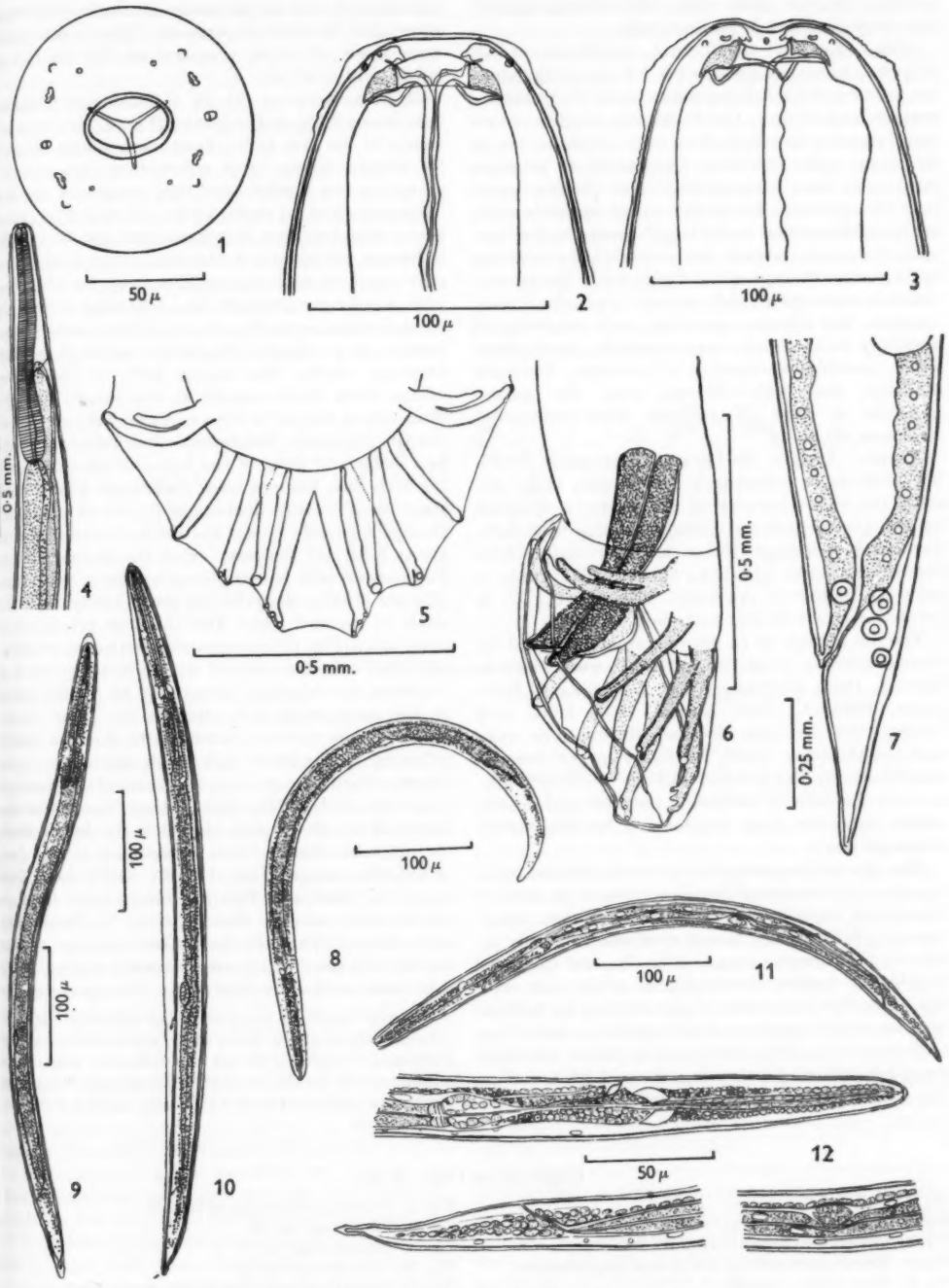
Dictyocaulus filaria (Rudolphi, 1809) Raillet & Henry, 1907 (Figs. 1–12)

This species is the oldest known lungworm of sheep. Of the several available historical reviews, that of Daubney (1920) is the most detailed. *D. filaria* is of world-wide distribution, and is one of the commonest lungworms of sheep and goats in the Levant. It is to be found in large and small bronchi, and it cannot be missed when the bronchi are opened because of its large size, and because in most cases several specimens, often dozens, may be found wriggling in thick, slimy pus, closing the infected site. In addition to adults the pus contains a multitude of its eggs containing first-stage larvae. In physiological saline adult worms remain alive for 5 days at room temperature, while in tap water they survive no longer than 48 hr.

Adult specimens are white and thread-like, and are enveloped in a teguminal sheath* which is only slightly wider than the body. The oral opening (Fig. 1) is oval, and its ventral rim appears thicker than the dorsal one. Slight depressions on both lateral sides of the perioral surface form dorsal and ventral lips (Fig. 3). Fourteen sensory papillae arranged in two circles, in addition to two amphids, surround the oral opening. The internal circle consists of six papillae which are so small that they may be seen only with difficulty. The external circle consists of eight much larger papillae arranged in sublateral pairs. The outermost of these papillae are globular while those near the amphids are elongate complicated structures.† The oral cavity is lined with a thick chitinoid capsule (Figs. 2, 3), 8–15μ deep, and 60–75μ wide externally. The capsular wall is almost triangular in optical section, but its thickness may vary. The oesophagus (Fig. 4) is club-shaped, with the posterior extremity twice as wide as the anterior one. The excretory pore is connected by

* For explanation of this term see Gerichter (1948*b*, p. 76).

† This arrangement of perioral papillae is probably peculiar to the genus *Dictyocaulus*. *D. viviparus*, occurring in this country in cattle and buffaloes, has been compared with *D. filaria*. The perioral structures of the two species appear to be essentially similar, except for the lateral perioral depressions which are deeper. The lips are thus more distinct in *D. viviparus* (Figs. 13–15).



Figs. 1–12. *Dictyocaulus filaria*. For legends see p. 168.

a short, narrow duct with two sausage-shaped excretory glands, 2·3–2·5 mm. long.

Male. Length 40–80 mm., maximum width 0·27–0·45 mm.; oesophagus 1·0–1·6 mm. long; copulatory bursa (Figs. 5, 6) prominent with the following arrangement of rays. The dorsal ray consists of two long branches united at their bases, each having at the distal extremity three papillae whose arrangement may vary considerably; the externo-dorsal rays are separate; the postero- and medio-laterals are fused along their entire length except at the very end; the antero-laterals are separate; the ventrals are united at their proximal third, while the ventro-ventrals are considerably shorter than the latero-ventrals. The spicules are stout, each consisting of a spongy stem slightly bent ventrally, having two wavy, alae-like membranes at the sides. They are identical, and 0·49–0·60 mm. long. The gubernaculum is oval, of spongy, semi-transparent structure, 60 μ long.

Female. Length 50–110 mm., maximum width 0·31–0·50 mm.; oesophagus 1·3–1·7 mm. long. Almost the entire interior of the worm is occupied by the genital tubules containing eggs with fully developed first-stage larvae at their distal ends. Size of the eggs: 129–138 × 86–94 μ. The vulga is near the middle of the body. The tail (Fig. 7) is pointed and is 0·39–0·45 mm. long.

The life history of *D. filaria* was investigated by several authors (Guberlet, 1919; Romanovitch & Slavine, 1914; Daubney, 1920; Hobmaier & Hobmaier, 1929*a*, *b*, 1930; Kauzal, 1933, 1934; and others), but the most detailed observations were made by Daubney (1920). The following are observations made by the present author, which not only confirm the data of Daubney, but also add details which have not been emphasized by the earlier investigators.

The eggs in the present study were obtained from the uteri of macerated females, cleaned in several changes of saline, and stored in tap water. Only ova with fully formed larvae were used, for only at this stage do larvae escape actively, and they are capable of further development, while ova containing earlier embryonic stages develop no further in water and disintegrate after some time. According to Orlov *et al.* (1937) larvae of *D. filaria* infesting sheep hatch during the passage through its intestine. In order to secure quantitative observations, hundreds of ova at the same developmental stage were used in each experiment. The observations were made at room temperature (21–25° C.), at 30° C., and at 37° C.

Room temperature (21–25° C.). At this temperature about 90 % of the larvae (Fig. 8) hatch in the course of the first 48 hr. They move about actively for several hours, after which they become progressively less motile, until they enter into the first lethargus after, at most, 24 hr. During this period the cuticle becomes detached from the body. The lethargus, which is not absolute, since a very slow movement of both extremities may be observed, lasts for longer than 24 hr., following which the second-stage larva (Fig. 9), enveloped in its former cuticle as a sheath, begins to move sluggishly. Daubney claims that about 10 % of the larvae escape from their sheaths at this stage; but this observation has never been confirmed in our experiments. Similarly, Daubney's observation that the first ecdysis of 10 % of the larvae is retarded until the fifth day, has not been confirmed. The second-stage larva remains unchanged for, at most, 24 hr.; though it usually enters the second lethargus after about 6 hr. At the same time, the second ecdysis commences, and is completed within a few hours. (Kauzal (1933), observed the second ecdysis on the sixth to seventh day.) The third or pre-infective stage larva (Fig. 10) emerges enclosed in two sheaths. Although the anatomy of the third-stage larva is constant, two kinds of larvae may be distinguished at the same stage and often in the same batch, namely, more active ones with both sheaths tightly adhering to the body and much less active ones, whose sheaths are swollen and distinctly separated from the body. The third stage also lasts for about 24 hr. at the end of which the larva sheds its external sheath, thus entering into the last or infective stage (Figs. 11, 12), called the 'final stage' by Daubney. This hatching occurs later in larvae with swollen sheaths than in those with tight sheaths. Thus the first infective larvae appear on the fifth day.* They are extremely active, being even more so than the first stage. At room tempera-

* Other observers have recorded different developmental periods of the larva of *D. filaria*: Hobmaier & Hobmaier (1929*b*) observed the infective stage after a minimum of a week, Kauzal (1933) after 6–7 days and Orlov *et al.* (1937) after 10–12 days in distilled water.

Legends to Figs. 1–12.

Fig. 1. Perioral structures.
Fig. 2. Anterior extremity, ventral view.
Fig. 3. Anterior extremity, lateral view.
Fig. 4. Anterior extremity, low-power magnification.
Fig. 5. Male bursa, opened.
Fig. 6. Male bursa, lateral view.
Fig. 7. Posterior extremity of female.
Fig. 8. First-stage larva.
Fig. 9. Second-stage larva.
Fig. 10. Third-stage larva.
Fig. 11. Infective larva, low-power magnification.
Fig. 12. Infective larva, high-power magnification.

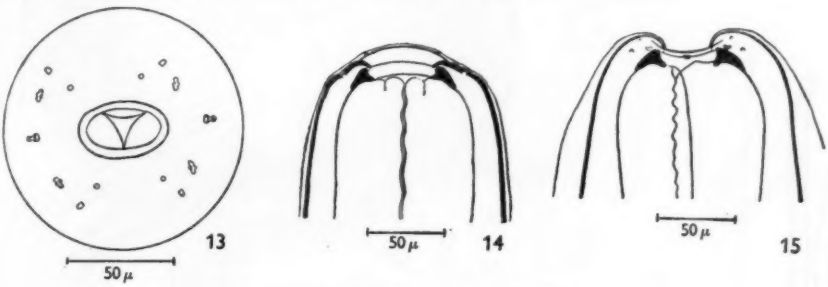
ture 2–8 % of the larvae cultured in tap water die during the period necessary for all individuals to reach the infective stage. Following this, the mortality rate gradually rises while the vitality of the larvae diminishes. However, after as long as 4 months, a few living, though inactive, larvae may still be found in the cultures.

Temperature 30° C. Hatching occurs sooner than at room temperature, about 60 % of the larvae having hatched after 24 hr. and practically 100 % after 48 hr. Development is also accelerated, a few larvae reaching the pre-infective stage on the third day and the infective stage on the fourth day. The mortality rate is higher than at room temperature.

Temperature 37° C. At this temperature almost 100 % of the larvae hatch after 24 hr. They develop to the second stage, but no further. Mortality is high and only a small percentage of the larvae survive to the fifth day, and none to the sixth day. Daubney (1920) also observed the failure of development to the third stage, and he assumed that the early death (100 % in 3 days in his experiments) was caused by bacterial growth. In order to determine whether the elevated temperature of the bacterial growth was responsible for the inhibition of development, larvae which had developed to the infective stage at room temperature were placed in clean tap water at 37° C., and the water was renewed daily. At the same time, a similar control batch of larvae was maintained at 21–25° C. No larvae survived for 5 days at 37° C., while all the controls were alive at this time. This shows that the infective larvae, which are known to differ from the earlier stages in their high resistance to desiccation and to low temperatures, are as sensitive to 37° C. as are other developmental stages; and also that bacterial growth is not the lethal factor. A temperature of 37° C. apparently kills most of the larvae during the first 3 days, and those remaining are unable to proceed with their development to the infective stage.

Observations were made on the development at room temperature of *D. filaria* larvae in sheep faeces free from *Dictyocaulus* larvae. Development proceeded along the same lines as in tap water, though it was slightly accelerated and some infective larvae were found a day earlier than in tap water control dishes. Kauzal (1933) claimed that third-stage larvae of *D. filaria* cast their first-stage sheath in faeces culture, but not in water. We have observed this process under both conditions.

It is noteworthy that larval development up to the third stage may occur without hatching even under abnormal conditions. In some instances such development was observed within the uterus of the intact female kept in saline, but the larvae never reached the infective stage.



Figs. 13–15. *Dictyocaulus viviparus.*

Fig. 13. Perioral structures.
Fig. 14. Anterior extremity, ventral view.
Fig. 15. Anterior extremity, lateral view.

Cystocaulus ocreatus (Raillet & Henry, 1907) Micaĉiĉ, 1939 (Figs. 16–28)

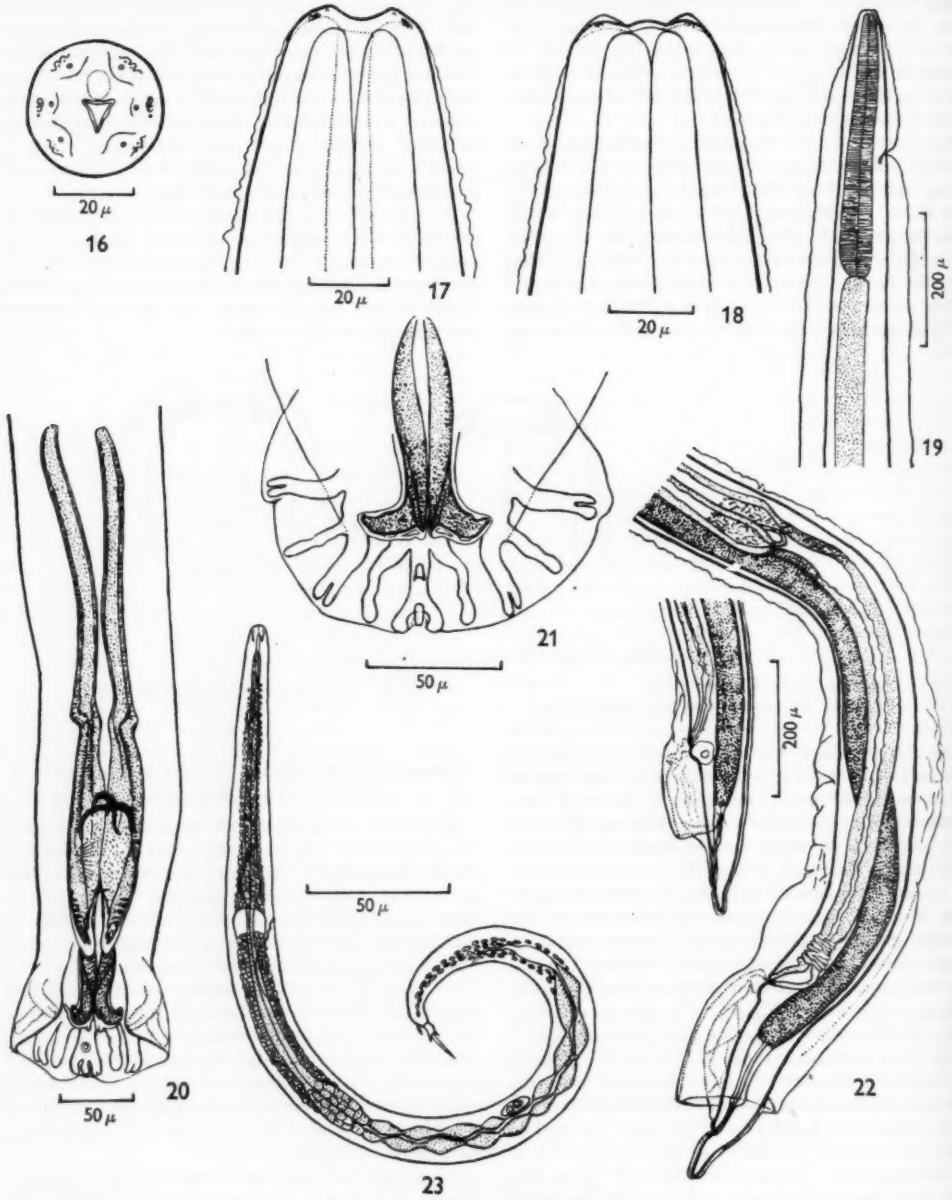
This is the only species of the genus *Cystocaulus*. It has been found in sheep and goats and in its adult stage is a parasite of the lung parenchyme. It occurs in Central and Southern Europe, North Africa, Armenia, Central Asia and parts of the U.S.S.R.

C. ocreatus usually occurs in the same lung in the following two well-differentiated stages:

(1) Sexually developed adults. Both males and females are found in the parenchyme, but never in the bronchi. Infected lobuli are swollen and infiltrated and are easily distinguishable from the surface of the lung by their pale colour. The diseased tissue contains numerous eggs and larvae. The adults are deeply imbedded in the tissue and only seldom can they be withdrawn intact.

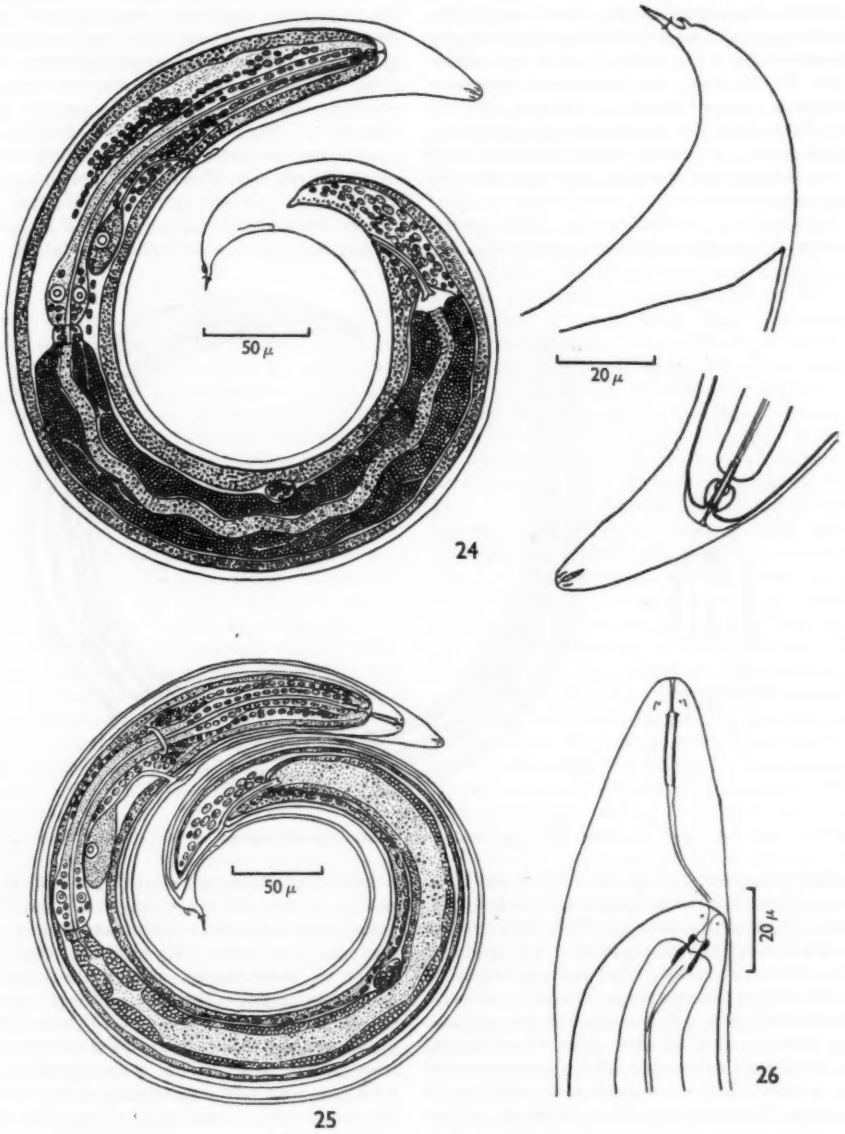
(2) Single males or single females, sometimes containing only a few undeveloped eggs, are usually located in dark nodules, *c.* 2 mm. in diameter, beneath the pleural surface of the lung. These worms are coiled, but they readily uncoil in saline and total specimens may easily be secured.

This species has been described by several investi-



Figs. 16–23. *Cystocaulus ocreatus.*

Fig. 16. Perioral structures.
Fig. 17. Anterior extremity, ventral view.
Fig. 18. Anterior extremity, lateral view.
Fig. 19. Anterior extremity, low-power magnification.
Fig. 20. Posterior extremity of male.
Fig. 21. Male bursa, opened.
Fig. 22. Posterior extremity of female.
Fig. 23. First-stage larva.



Figs. 24–26. *Cystocaulus ocreatus.*

Fig. 24. Second-stage larva.

Fig. 25. Third-stage larva.

Fig. 26. Anterior extremity of third-stage larva, high-power magnification.

gators, mostly fragmentarily, and under several names. Micačić (1939), finally corrected the name and revised the type specimens. Dougherty & Goble (1946) enumerated all the synonyms of this species. Anatomical features of adults have recently been described by Lutz (1926), under the names *Strongylus* Utz-Lydtin and *Strongylus* (*Synthetocaulus*) *nigrescens ovis* Utz-Jerke. Schultz, Orlov & Kutass (1933), drew the bursa and gubernaculum, and Schulz & Boyev (1940*a*), and Joyeux & Gaud (1946), re-investigated the anatomy and followed the life history.

The following is a new description of this species based on local material. The body is thread-like and brown. The oral opening (Figs. 16–19) is central and triangular. There is no oral capsule and no true lips. There are four sublateral larger perityls (for explanation of this term see Gerichter, 1949, p. 251) and two lateral lower ones. A rounded depression occurs on the dorsal and ventral perioral ridge. The internal circle of perioral papillae consists of six minute papillae, and the external one of four pairs of sublateral papillae and two extremely small latero-ventral papillae, a total of sixteen papillae, in addition to two amphids. The oesophagus is cylindroid, a little wider in its posterior than in its anterior part. The excretory pore is just behind the middle of the oesophagus and the deirids a little behind it, in the form of elongated papillae. The teguminal sheath is wide, with many folds along the entire body.

Male. Length 31–45 mm. (Schulz & Boyev measured 18–24 mm.); maximum width 0·06–0·08 mm.; oesophagus 0·36–0·39 mm. long (ratio to the length of the body *c*. 1:100). The intestine is of uniform width throughout. The bursa (Figs. 20, 21) is not distinctly divided into lobes. The dorsal ray is of varying width and its distal third is split into three usually equal branches, though in some specimens the tips of the side branches are slightly split while the middle branch may vary slightly in shape and position. A large elongate papilla may be attached to the middle of the stem of the dorsal ray. The externo-dorsal rays vary in width. The postero- and medio-lateral rays are united along three-quarters of their length and are widely separated from the antero-lateral ray. The ventral rays are united along three-quarters of their length. The spicules are 0·29–0·37 mm. long, each consisting of two parts, a proximal almost cylindroid portion, joined distinctly with the distal portion, which is lance-shaped with a thick outer ridge. At the distal end of the spicules there are four to six denticles which are directed either posteriorly or anteriorly and the entire region is covered by a thin, transparent layer. The gubernaculum is 0·12–0·15 mm. long and is composed of three elements—the capitulum, corpus and crura. The capitulum consists of two unequal sclerotized hooks crossing each other and is connected with the crura by the corpus which is a transparent membrane, a little darker at sides. The crura consist of two dark-brown, boot-shaped structures with the distal tips directed dorsally, and not ventrally, as stated by Schulz & Boyev (1940*a*). The distal part of the crura is

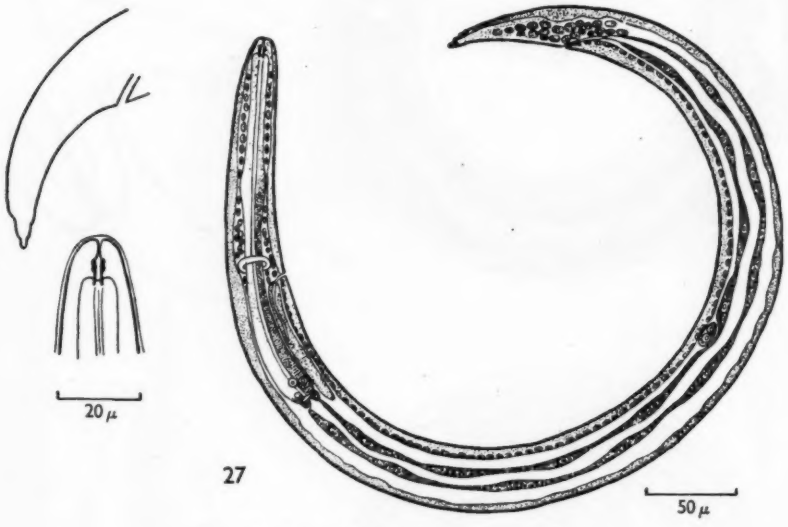


Fig. 27. *Cystocaulus ocreatus*. Infective larva.

covered with a transparent layer. No sclerotized arches, like those in the drawing of Schulz, Orlov & Kutass (1933), were observed in the local specimens. (Folds of the homogeneous layer of the crural extremities may have been confused with arches.)

Female. Length 55–95 mm., width 0·99–0·12 mm.; oesophagus 0·23–0·50 mm. long; vagina 0·5–1·0 mm. long. The vulva is 0·15–0·26 mm. from the anus. The anus is 0·04–0·08 mm. from the tip of the tail. The teguminal sheath expands at the level of the vulva, forming a bell-shaped screen (Fig. 22) not reaching to the anus over the posterior extremity of the worm. In the description of Schulz & Boyev this bell was apparently taken for a provagina.

Life history

The first attempt to investigate the life history of *C. ocreatus* [*C. nigrescens*] was that of Schulz & Boyev (1940*b*), who unsuccessfully tried to infect several species of terrestrial molluscs in Kazakhstan. Although the larvae penetrated into the molluscs, no further development followed over a period of 20 days. These experiments were continued by Boyev & Wolf (1940) who obtained development in molluscs of two species of larvae, one of which was provided with a tail spine. No further determination of the species of these larvae was indicated and no figures were given. Since two of the three species with which Schulz & Boyev experimented had a tail spine, it is impossible to determine whether *C. ocreatus* was among them. Lately Joyeux & Gaud (1946) studied the life history of this species, but their work is incomplete and it is not substantiated by drawings. For instance, no tail spine is mentioned in the description of the first-stage larva, a brown rigid casing is described in the infective stage, but was never observed by the present author.

Ova of *C. ocreatus* are laid in an early stage of embryonic development in the tissues of the host's lung. They are narrow and oval, with both ends equally rounded. Their dimensions are 80–90 × 50–60 μ. They are found in large numbers in all stages of development in the diseased foci of the lung, together with first-stage larvae which have hatched *in situ*.

First-stage larva (Fig. 23): length 390–420 μ, maximum width 18–19 μ. The cuticle is distinctly striated. The oral opening is central and leads, through a short narrow tubule, into a cylindroid oral capsule 7–8 μ high. The oesophagus is rhabditoid, with a distinct metacorpus and bulbus, its length being about half that of the larva. The excretory pore and the nerve ring are situated at the level of the beginning of the isthmus. In the freshly hatched larva the intestine is of granular structure, but after prolonged maintenance in water the granules disappear and trapezoid cells become distinct. The genital primordium is situated near the middle of the intestine and usually contains two cells. The distance between the anus and the tip of the tail is 40–45 μ. The tail tip is very characteristic. It is slightly bent dorsally and two constrictions at its extremity form two appendages one behind the other, the proximal one being thicker and shorter and the distal one spine-shaped and longer. A short, bent spine occurs dorsally, just before the proximal appendage, and two minute denticles are found at the junction of the two appendages. The first-stage larvae remain alive in water for several months. Very active at first, their vitality gradually diminishes.

Experiments on the artificial infection of terrestrial snails and slugs were carried out in order to follow the further development of the larvae. A number of these hosts proved susceptible to infection and observations have been made on the entire life history of the parasite. The following is a description of its development under optimum conditions, i.e. in the most suitable mollusc and at a temperature of 20–30° C.

The first-stage larva reveals a particular affinity for the snail when it is brought into contact with its foot. It makes vigorous efforts to penetrate the tissues, and usually it soon succeeds. During the first 4 days the larva undergoes no structural change. The first distinct change observed is its growth and the appearance of numerous food granules of varying size in the intestinal cells. The growth of the larva continues up to the twelfth day. The first lethargus then begins and the first ecdysis is completed within 1 or 2 days thereafter. The second-stage larva (Fig. 24, Pl. III, fig. 28, *a*) is 610–630 μ long and 46–48 μ wide. The oral aperture opens into a narrow oral capsule about 12 μ long. The oesophagus extends through about one-third of the length of the body. The excretory duct is connected with an oval excretory gland situated above the bulbus. The intestine is conspicuous by its width and dark tint due to food granules which fill its cells. The distance between the anus and the tip of the tail is 50–55 μ. The tip of the tail is conoid and devoid of any appendages. The second stage lasts for 1–2 days, followed by the second lethargus. One to 2 days later (i.e. 15–17 days after penetration into the snail) the larva appears with two detached skins, i.e. in its third or pre-infective stage (Fig. 25, Pl. III, fig. 28, *b*). It is noteworthy that at this stage the second detached skin may be seen to consist of two sheaths, which are separated from each other particularly at the posterior extremity of the larva. For this reason three instead of two sheaths may clearly be seen at this point.

The third-stage larva is longer (700 μ), and narrower (36–38 μ) than the preceding stage. The oral opening (Fig. 26) leads through a narrow passage into a cylindroid oral capsule and further

into the oesophagus, which occupies less than one-third of the body length. Intestinal cells contain food granules, but their number is much reduced. The tip of the tail is conical and is 40–42 μ from the anus. The larva of this stage is more active in water than the second-stage larva.

Two days after the completion of the second ecdysis (18–19 days after penetration) the larva changes into the infective stage (Fig. 27, Pl. III, fig. 28, *c*) without shedding its skin. It is a little longer and slenderer than the pre-infective stage, and its dimensions are 750–790 μ by 36–37 μ. The oesophagus differs from that of the preceding stage in that it has no nuclei in the procorpus, while fine, greenish granulation appears in the sublateral segments of the bulbus. The intestinal wall contains no food granules, and after a prolonged period its structure becomes apparent and it appears to consist of eight spindle-shaped cells. The tip of the tail has a minute, button-shaped appendage and the distance between it and the anus is 54–62 μ. As contrasted with the pre-infective stage, the detached skins of the infective larva become extremely fragile and they easily break when the examined snail tissue is lightly pressed between slides. The larva thus freed of the sheaths is very active in water.

The above type of larval development of *C. ocreatus* has been observed in the following species of molluscs:* *Chondrula septemdentata*, *Helicella barbesiana*, *H. vestalis joppensis*, *Helix cavata*, *Levantina caesareana*, *Levantina hierosolima*, *Monacha syriaca*, *Theba pisana*, *Retinella nittellina Agriolimax* sp. and *Limax flavus*. These hosts may, however, be considered appropriate or inappropriate,† depending on the length of time necessary for larval development to the infective stage. While in appropriate species of molluscs (both species of *Helicella*, *Monacha* and both species of *Limax*) full development at optimum temperature took 18–19 days, in the inappropriate species (both species of *Levantina*) several months were required under identical conditions. The remaining host species required intermediate periods to complete larval development.

The above rate of larval development occurs at the optimum temperature (20–30° C.) At lower temperatures development was retarded and at 4–8° C. it was so slow that after 5 months the larvae were not yet ready for the first ecdysis. Slowly developing larvae, when they are transferred to the optimum temperature after any period of retardation by reduced temperature, at once resume normal development as if they had not been maintained at low temperatures; and they complete their development in a minimum period. The controls, left at refrigerator temperature (4–8° C.). remain undeveloped. These observations confirm the explanation of Schulz & Boyev (1940*b*), of their failure to observe larval development of *Cystocaulus ocreatus* beyond the first stage in Central Asiatic molluscs over a period of 20 days. Apparently the low temperature of September in Kasakhstan was the cause of the retardation.

In an experiment to complete the study of the life history of *C. ocreatus*, two suckling kids, not hitherto exposed to field conditions and with no helminthic infestations at several successive examinations, were each fed two teased feet of *Monacha syriaca* mixed with milk, containing altogether about 100 infective larvae of *Cystocaulus ocreatus*. Accidentally, both kids contracted undetermined pustulous disease 2 weeks later and one of them died during the following week. No worms were found in its lungs on dissection. The second kid recovered and, on the 42nd day, the first larvae of *C. ocreatus* appeared in its faeces. Their number increased daily. Three weeks later, i.e. 9 weeks after the infective feed, the kid was sacrificed. The distal tips of both lungs were indurated and infiltrated and contained adult males and females as well as larvae and ova of *C. ocreatus*. It is noteworthy that no nodules were found containing developed worms, which are characteristic in natural infections.

Muellerius capillaris (Mueller, 1889) Cameron, 1927 (Figs. 29–39)

The systematic position of this species has been much debated and it is therefore listed in the literature under no less than ten different names. Dougherty & Goble (1946) revised its nomenclature and gave a complete list of its synonyms and Joyeux & Gaud (1946) reviewed its history. Most of the authors dealing with this species determined its systematic position incorrectly, because details of the bursa were unrecognized. Cameron (1927) first applied the modern approach to its systematic position. He recognized its peculiarity and created for it the new genus *Muellerius*. He did not, however, recognize the true structure of the bursa, which he erroneously called genital papillae. Recent investigators have recognized some of the rays, but the genus *Muellerius* has been regarded by most of them as belonging to taxonomic groups devoid of bursa (Filaroidinae, Pseudaliinae), except Dougherty & Goble (1946) who rightly placed it in the Protostrongylinae. The latter authors, however, proposed to substitute the name *M. minutissimus* for *M. capillaris*, on the assumption that the species is identical with *Strongylus minutissimus* Megnin, 1878. The present author cannot accept this argument. No anatomical details, except the length of the adults, agree with the characteristics in Megnin's description of *Muellerius capillaris*. Indeed some of the charac-

* The author is indebted to Prof. G. Hass of the Department of Zoology, Hebrew University, for the identification of the snail species.

† See Gerichter (1948*a*, p. 110).

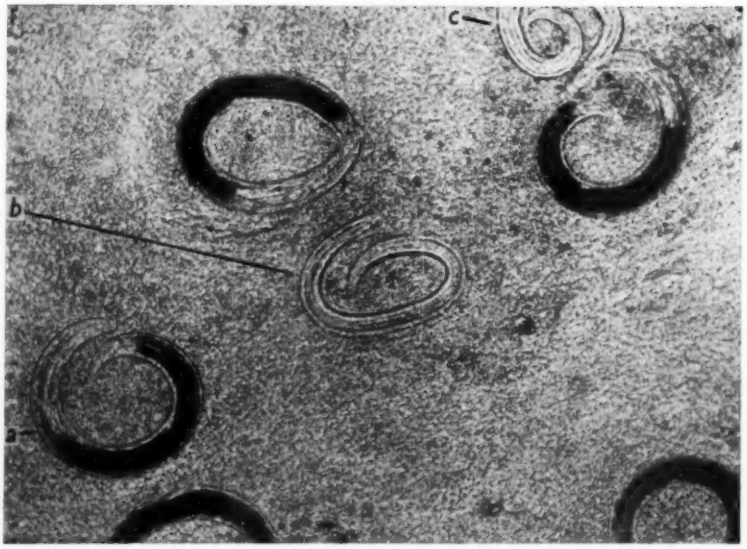


Fig. 28. *Cystocaulus ocreatus*. Larvae in tissues of snail (artificial infection): *a*, second stage; *b*, third stage; *c*, infective stage.

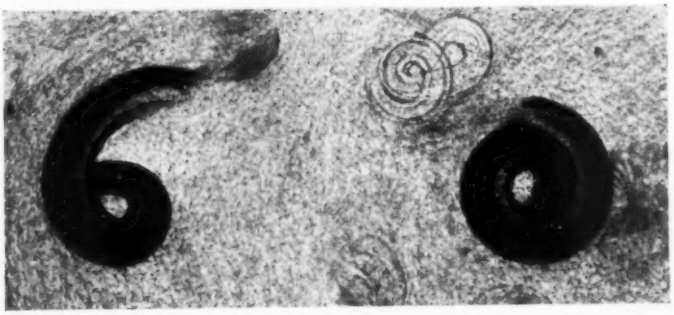
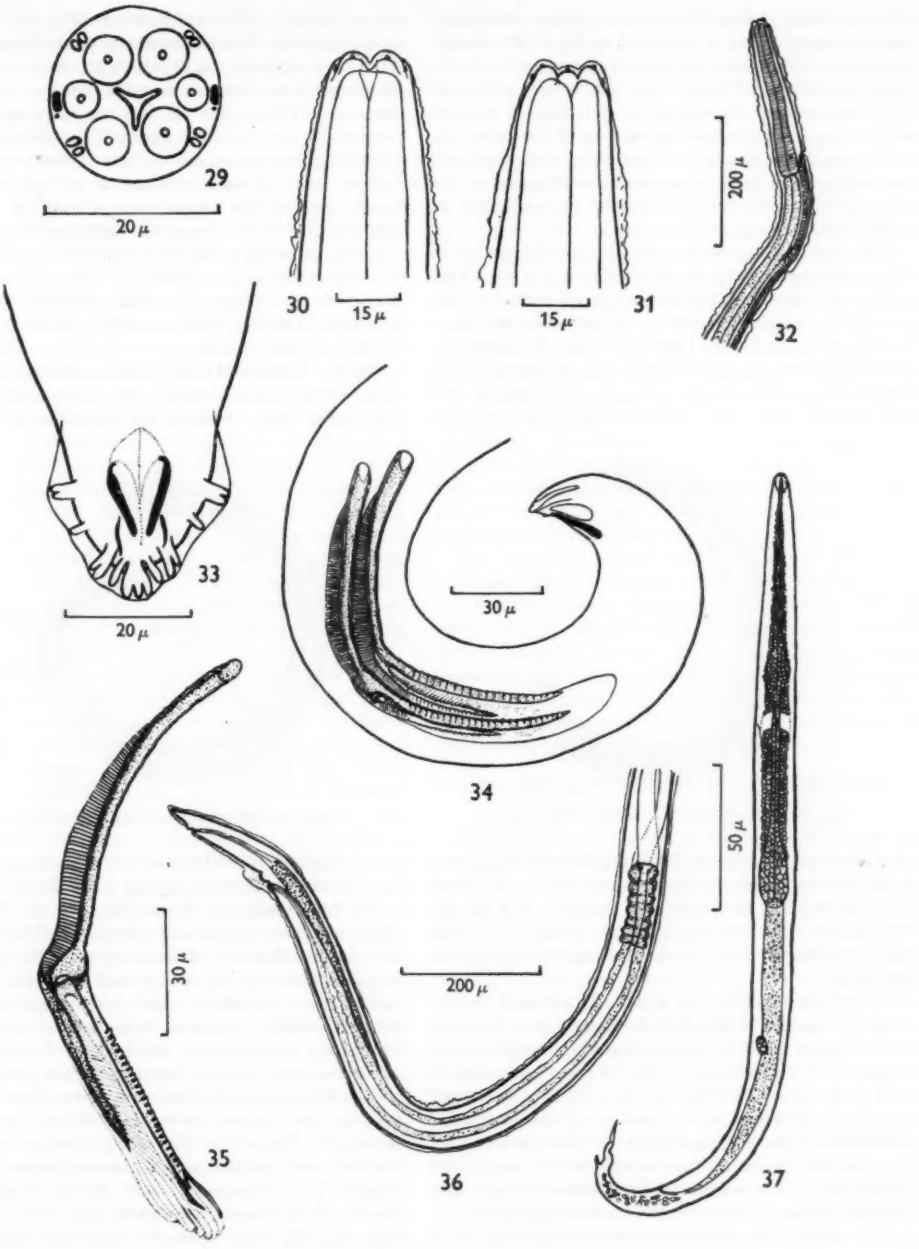


Fig. 50. *Protostrongylus rufescens*. Two castings and one freed infective larva in tissue of snail (artificial infection).

Fig. 29.
Fig. 30.
Fig. 31.
Fig. 32.
Fig. 33.



Figs. 29–37. *Muellerius capillaris.*

Fig. 29. Perioral structures.
Fig. 30. Anterior extremity, ventral view.
Fig. 31. Anterior extremity, lateral view.
Fig. 32. Anterior extremity, low-power magnification.
Fig. 33. Male bursa, opened.
Fig. 34. Posterior extremity of male, showing spicules.
Fig. 35. Separate spicule.
Fig. 36. Posterior extremity of female.
Fig. 37. First-stage larva.

teristics differ widely. Thus, for example, in Megnin's paper a large bursa is depicted in fig. 3 (Pl. 2) and rays are indicated in the text. The vagina is shown to be equatorial. The width of the female is four to five times that of *M. capillaris*. No mention is made of the very characteristic spiral coils of the male, etc. It seems that Megnin's description has confused several species, and it cannot therefore serve for species identification. It should be regarded as species *inquirenda*.

The first designation of *M. capillaris* is as *Nematoideum ovis* (*pulmonale*) Diesing, 1851. This name was proposed for the unnamed larvae of the lungworms of English sheep, which were described by Sandie & Padley in 1849 but were left unnamed. It is fairly certain that *Muellerius capillaris* is the commonest lung parasite of sheep in England, and its larvae have the peculiar wave-shaped tail

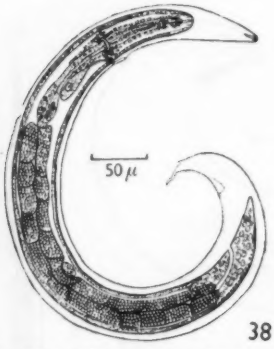


Fig. 38. *M. capillaris*. Second-stage larva.

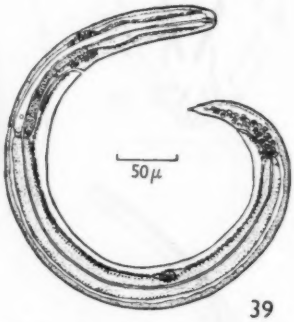


Fig. 39. *M. capillaris*. Infective larva.

described by Sandie & Padley (1849).* The name proposed by Diesing cannot, however, be accepted because it is not the name of a parasite, but simply designates a *Nematoideum* of the sheep lung. The name *capillaris*, next in priority, has therefore to be accepted.

Muellerius capillaris is widely distributed, occurring in Europe, North-Africa, Central and Western Asia. It is common in sheep and goats throughout the Levant. It localizes only in the lung parenchyme and never occurs in the bronchi. It is often found together with *Cystocaulus ocreatus*. It causes lobular infiltrated indurations, similar to those induced by *C. ocreatus*, and eggs, larvae and adults are found imbedded in the tissues in these diseased foci. The following description is based on local material.

The adults are filiform and are enclosed in a teguminal sheath with numerous transverse folds along the body. The oral aperture (Fig. 29) is central and triangular. There is no oral capsule (Figs. 30, 31). There are six perityls, of which the four sublaterals are larger than the two laterals. The row of minute sensory papillae, one on the top of each perityl, constitute the internal circle. The external circle consists of four pairs, each of which is situated at the lateral base of each sublateral perityl, and two barely perceptible latero-ventral papillae situated just ventral to the amphids. Altogether there are sixteen perioral papillae in addition to two amphids. The oesophagus is cylindroid (Fig. 32). A long excretory gland opens near the posterior end of the oesophagus and, at the same level, there is a pair of minute conical deirids.

Male. Length 11–13·5 mm.; maximum width 0·032–0·035 mm.; length of oesophagus 0·18–0·22 mm.; ratio between the oesophageal and the body length, 1:70. The posterior extremity is curled into eleven to thirteen spring coils. There is a very small but complete bursa (Fig. 33), *c.* 30 μ long, always folded inward and not discernible in profile. Due to the elasticity of the body coils, the bursa can be observed only on an excised body tip. All the bursal rays are short, the dorsal ray being the longest, with a narrow base and a wide distal extremity divided into three short branches. The externo-dorsal branch is separate; the postero- and medio-laterals are united along two-thirds of their length; the antero-laterals are short and widely separated from the preceding ones; while the ventrals are united along three-quarters of their length. Two sclerotized rods, 13–14 μ long, in the centre of the bursa, provided with thin, proximal tips slightly bent inward, may be regarded as a gubernaculum. Posterior to these rods a pair of much smaller, thin, sclerotized bows may be seen. The spicules (Figs. 34, 35) are identical, 0·14–0·16 mm. long, each consisting of a proximal stem and two unequal branches, of which one is three-

* It should be recalled that among the sheep lungworms only the larvae of the two species *M. capillaris* and *Cystocaulus ocreatus* are known to have a waved tail. The latter does not occur in England.

quarters as long as the other. The stem has a transversely-striated dorsal alum, both branches of which have a denticulated edge (though this is more prominent on the longer branch) and two thin appendages near its end. The branches are connected by a thin, wrinkled membrane.

Female. Length 20–22 mm.; maximum width 0·04–0·05 mm.; oesophagus 0·20–0·24 mm. The vagina, 0·85–0·90 mm. long, has a prominent sphincter (Fig. 36) at the junction of the uteri. The distance between the vulva and the anus is 0·11–0·13 mm. and the distance between the anus and the tip of the tail is 0·04–0·05 mm.

The life history of this species is similar to that of *C. ocreatus*. Schlegel (1899) unsuccessfully attempted to infect sheep by feeding them first-stage larvae and concluded that intermediate hosts, namely snails, earthworms or insects, are necessary. This assumption was supported by Hobmaier & Hobmaier (1929*a*), who observed the development of larvae of this species through the infective stage in a number of land snails and slugs. Bohn (1937), apparently quoting a paper which he does not identify, asserted that this species develops into adults in sheep within 10–12 weeks. Davtian (1937) and Joyeux & Gaud (1946) established new snail hosts for this species for Armenia and North Africa, respectively.

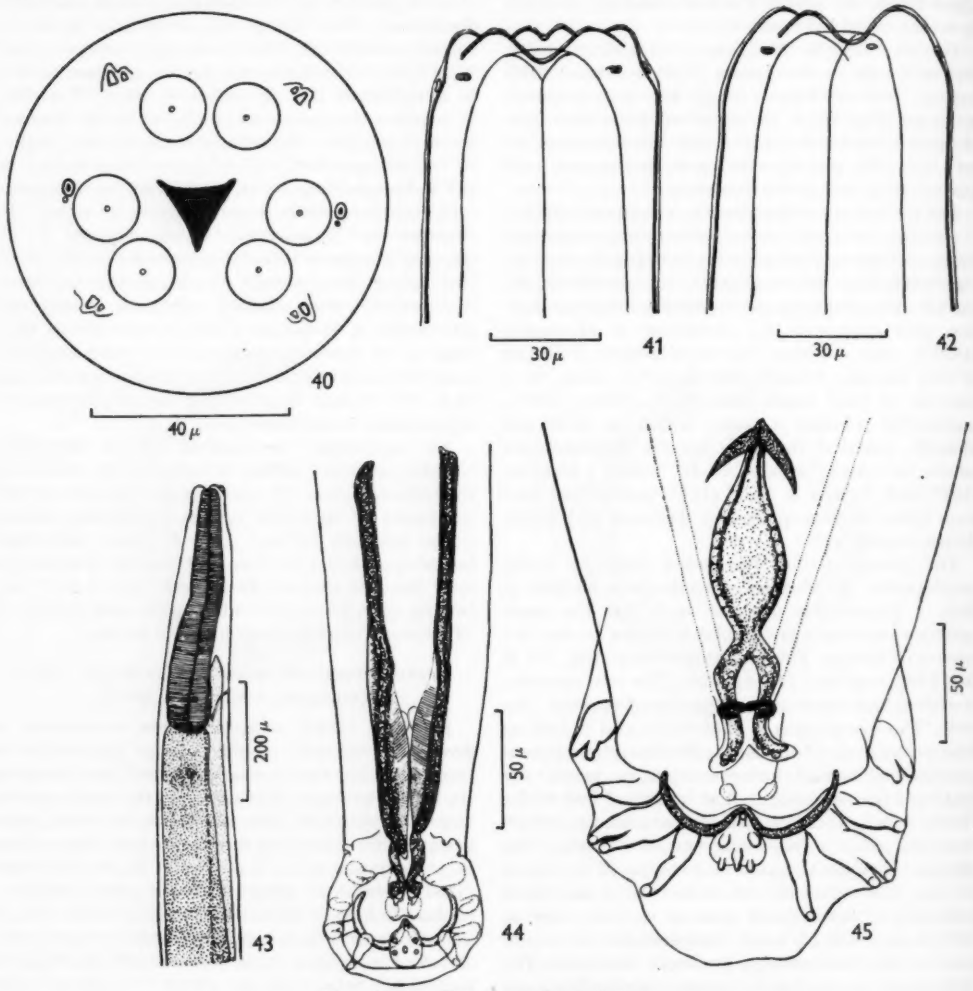
The present author has found that the larval development of *Muellerius capillaris* is similar to that of *Cystocaulus ocreatus*, and that the same molluscan species have similar affinities to the two species of larvae. The first-stage larva (Fig. 37) is 300–320 μ long and 14–15 μ wide. The oral aperture is central and opens into a cylindroid capsule, 10 μ wide. The oesophagus is rhabditoid and is half as long as the body of the larva. The excretory pore is situated just behind the metacorpus, i.e. behind the middle of the oesophagus. The intestinal wall of the freshly hatched larva is rich in food granules, which disappear after a free-living period in water. The distance between the anus and the tip of the tail is 40–45 μ. The tip of the tail is undulated and bears a dorsally situated, small spine on its base. Larvae survive in water at room temperature for several months, but their activity gradually decreases. The first ecdysis is completed in susceptible molluscs and at the optimum temperature on the 28th day. The second-stage larva (Fig. 38) is 550–560 μ long and 37–38 μ wide. The oesophagus is less than a third the length of the body. The oval excretory gland lies above the bulbus and opens approximately at the level of the middle of the oesophagus. The intestine is very dark, due to the food granules tightly filling its cells. The tail is 48–50 μ long, conical and devoid of any appendages. The second stage lasts for 2 days and, after a lethargus of 1 or 2 days, the third or pre-infective stage commences. The length of the third-stage larva is 600–620 μ and it is 36 μ wide. The oral capsule is somewhat wider and shorter than in the preceding stage. The oesophagus occupies a third of the body length. The number of food granules in the intestinal cells is markedly diminished. The tail is 50 μ long and its tip is conoid, smooth and without any appendages. After 2 or 3 days the larva changes its appearance and is transformed into an infective larva (Fig. 39). It acquires the properties of similar larvae of allied metastrongylids, i.e. its shed sheaths become fragile, its oesophagus is devoid of granulation except in the subventral segments, food granules disappear and the larva becomes more active in water. In contrast to *C. ocreatus*, which requires 18–19 days for complete larval development in the snail, the larval development of *Muellerius capillaris* requires 35 days under optimum conditions. (Hobmaier & Hobmaier (1929*a*) have stated that only 12–14 days are required.) At lower temperatures larval development is markedly retarded and at 4–8° C. it may last for 4½–5 months, even in an appropriate intermediate host.

An experiment was carried out to determine whether infective larvae developed into adults in the definitive host. A suckling kid was fed several specimens of *Monacha syriaca* containing several dozen infective larvae. After 6 weeks, first-stage larvae appeared in the faeces of the kid. A fortnight later the kid was sacrificed and typical foci containing eggs, larvae and adult males and females of *Muellerius capillaris* were found in its lungs.

Protostrongylus rufescens (Leuckart, 1865) Kamensky, 1905 (Figs. 40–50)

Leuckart (1865) established the occurrence of *Strongylus rufescens* in sheep lungs apparently in Germany. His description presented in a footnote (p. 200) is so vague that not even the genus can be clearly determined. This name has, however, been accepted for the sheep-lung nematode believed to be the commonest in Europe and Raillet & Henry (1907) decided to assign it to the genus *Synthetocaulus*. Leiper, in 1926, demonstrated that the latter generic name is a synonym of *Protostrongylus* and Leuckart's species thus became *Protostrongylus rufescens*. Schulz *et al.* (1933) concluded that Leuckart's original description is insufficient for the determination of a species and that it is impossible to judge which of several lung nematodes now known as sheep parasites Leuckart was actually describing. They therefore concluded that a new specific name should be given to the common European *Protostrongylus* of sheep (still called by them *Synthetocaulus*), and they proposed the name *kochi* in honour of A. Koch, who in 1883 gave the first recognizable description of this species. However, this view has not been shared by more recent authors, such as

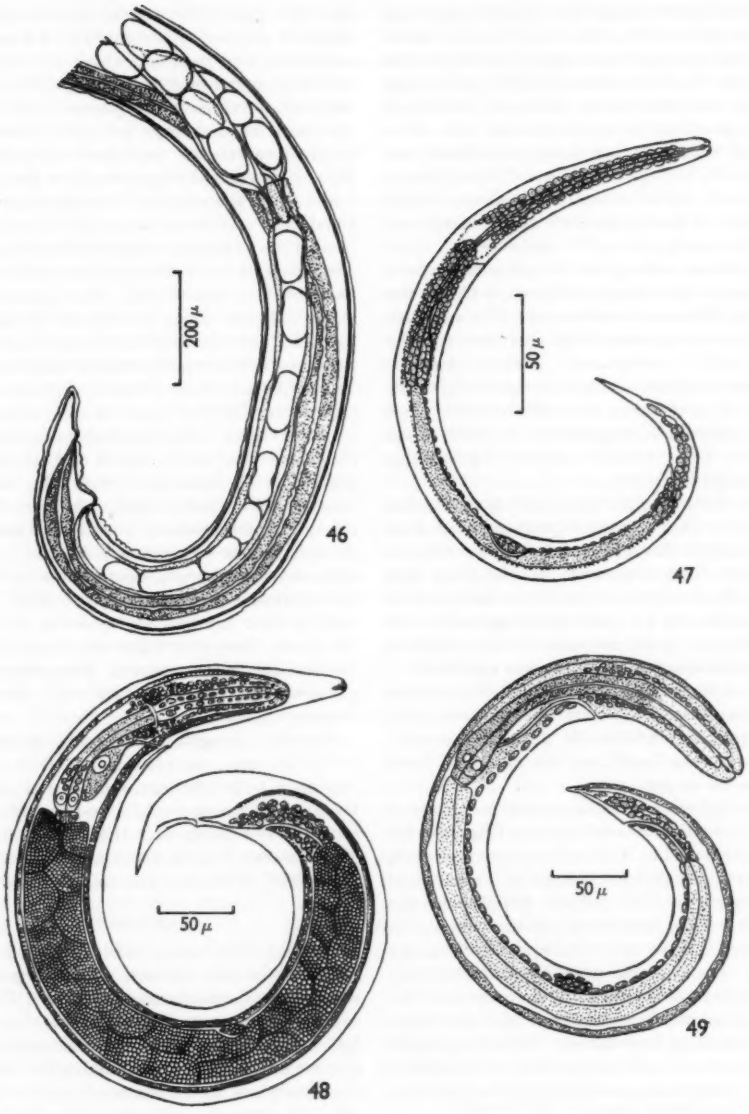
Dougherty & Goble (1946) or Joyeux & Gaud (1946), who continued to use the specific name *rufescens*. The present author adopts the latter version, although, in a previous publication (1948*a*), *Protostrongylus kochi* has been quoted instead. The cause of that nomenclatural conception was the inaccessibility of Leuckart's paper, while Leuckart's inadequate description of the adult has been known only from a quotation by Lutz (1926). Later a microfilm of Leuckart's paper was obtained and a description of an important detail was revealed, which proved the identity of Leuckart's species with that called *Synthetocaulus kochi*. Leuckart mentioned as a peculiarity of his species the straight tail of its first-stage larva. Such a tail is known only in *Protostrongylus*, while other species of sheep lungworms have a tail either waved or provided with constrictions or spines or both.



Figs. 40–45. *Protostrongylus rufescens*.

Fig. 40. Perioral structures.
Fig. 41. Anterior extremity, ventral view.
Fig. 42. Anterior extremity, lateral view.
Fig. 43. Anterior extremity, low-power magnification.
Fig. 44. Posterior extremity of male.
Fig. 45. Male bursa opened, showing gubernaculum.

Several authors have described *P. rufescens* in detail, chiefly under the name of *Strongylus rufescens* or *Synthetocaulus rufescens*. Full synonyms of this species were given by Dougherty & Goble (1946).



Figs. 46–49. *Protostrongylus rufescens.*

Fig. 46. Posterior extremity of female.
Fig. 47. First-stage larva.
Fig. 48. Second-stage larva.
Fig. 49 Infective larva.

The most recent description is that of Joyeux & Gaud (1946). Most modern authors regard *Protostrongylus rufescens* as a specific parasite of sheep and goats and assume that this species does not occur in hares and rabbits, for which hosts at least three peculiar species have been described. This view is not shared by Raillet & Henry (1907), who were quoted by several subsequent authors. Joyeux & Gaud (1946), revived the early theory in a compromise. They consider that European hares and rabbits harbour a biological variety of *P. rufescens*, called by them *P. rufescens* var. *cuniculorum*, which cannot develop in sheep, as well as an additional species of the same genus, *P. pulmonalis*. Conversely, the sheep variety of *P. rufescens* cannot develop in hares. According to Joyeux & Gaud, the morphological difference between the two varieties of *P. rufescens* is not greater than that between the hare variety and *P. pulmonalis*. If these observations are correct, all three forms, viz. *P. rufescens* from sheep, *P. rufescens* var. *cuniculorum* and *P. pulmonalis*, should be regarded as varieties of the same species. The present author leaves this nomenclatural problem open.

The species occurring in sheep and goats in the Near East is in the meantime regarded as *bona species* and is called *P. rufescens*. *P. rufescens* is not the commonest lung nematode of sheep in any country. On the contrary, it has never been found in some countries, as, for instance, England or the Moroccan Plateau. In the Levant it is less common than *Cystocaulus ocreatus* or *Muellerius capillaris*.

Although existing descriptions of this species contain sufficient details for its identification, the author has gathered additional data and a new, complete description based on the study of local material is therefore presented.

The body is filiform in both sexes and is contained in a teguminal sheath. The oral opening (Figs. 40–42) is central and triangular. The oral capsule is absent. Six perityls are arranged in three pairs—one dorsal and two subventral. The minute papillae in the centre of each perityl constitute the internal circle, while the external circle of perioral papillae consists of four pairs of sublateral papillae and two tiny latero-ventral ones, in addition to two amphids. The oesophagus (Fig. 43) is cylindrical and its hind-portion is somewhat broadened. Deirids and the excretory pore are situated at the level of the widest portion of the oesophagus, and the pore is connected with a narrow excretory gland.

Male. Length 22–46 mm., maximum width 0·13–0·17 mm. The oesophagus is 0·24–0·37 mm. long, and its ratio to the body length is *c.* 1:100. The bursa (Figs. 44, 45) is well developed and has the following ray structure. The dorsal ray is almost globular, its ventral side bearing six papillae which, in an entirely opened and flattened bursa, are seen to be disposed in four quincuncial rows in the following manner. At the proximal edge of the ray there is one elongated papilla, followed by two globular ones. The fourth and longest papilla is situated almost in the middle of the ray and the remaining two papillae, which are the smallest, are at the posterior edge of the ray. The externo-dorsal rays are separated. The postero- and medio-lateral rays are fused except for their distal third. The antero-laterals are separated from the preceding rays. The ventral rays are short and wide and are fused along the proximal two-thirds of their length. Inside the posterior extremity of the body, at the base of the bursa, is a sclerotized structure, the arcus, consisting of two bows united approximately at the base of the dorsal ray. The spicules (Fig. 44), 0·24–0·34 mm. long, are almost straight and their distal halves bear two membranous pectinated alae forming grooves which communicate with each other. The gubernaculum consists of three parts, the capitulum, corpus and crura, whose length is 0·12–0·17 mm. The capitulum consists of four elements—two wide ventral and two narrow dorsal parts. It is connected with the corpus, which consists of a sclerotized, piriform frame and a granular membranous sheath between them, although this is sometimes thin and indistinct. The crura are dark-brown, boot-shaped prolongations of the corpus, whose tips are provided with several, usually four knobs. At the level of the middle of the crura there is a telamon, shaped like a 3 with its concave sides ventral. The surface of all the gubernacular elements may vary from smooth to rough or nodded.

Female. Length 30–65 mm., maximum width 0·15–0·29 mm., oesophagus 0·35–0·38 mm. long. The vagina is 1·10–1·30 mm. long and is separated from the uteri by a strong sphincter (Fig. 46). The distance between the vulva and the anus is 0·12–0·25 mm. The distance between the anus and the tip of the tail is 0·07–0·13 mm. The tail is conoid.

Life history

A. & M. Hobmaier, 1930*a*, first investigated the life cycle of this species, which they called *Protostrongylus* (*Synthetocaulus*) *rufescens*. The Hobmaiers carried out experiments with several species of snails but succeeded in obtaining full development in three species only of the genus *Helicella*. In these intermediate hosts the larvae reached the infective stage after 12–14 days. Infection of the final host was not recorded. Davtian (1937) studied the development of a species which he regarded as *Synthetocaulus rufescens*. He found that females of this species laid eggs not fully developed in saline at 28–30° C. and that, 24–36 hr. later, first-stage larvae hatched from them. These larvae had a dorsal spine at the tip of the tail. They developed to the final stage in

thirteen species of snails and slugs of different genera within 36–65 days. As these data do not correspond to the observations of the Hobmaiers, Davtian concluded that the species investigated by the Hobmaiers was not identical with the one he himself studied and that the Hobmaiers' determination was erroneous. Davtian did not attempt to infect the final host. Evidently Davtian's determination was itself incorrect. The present author has observed that the first-stage larva of *Protostrongylus rufescens* has a smooth tail, as shown by the Hobmaiers, and that its eggs are incapable of developing *in vitro*.

Joyeux & Gaud (1946) investigated the life cycle of a species which they call *P. rufescens*. Although these authors distinguished between two biological varieties of this species, one from sheep and the other from hares and rabbits, they observed no difference between the varieties in the course of their development in snails. As in the case of *Cystocaulus ocreatus*, these authors regarded the immature larva within the egg shell of *Protostrongylus rufescens* as the first developmental stage and they consequently designated subsequent stages as second, third and fourth. The following is a description of the life history of *P. rufescens* as observed by the present author.

The first-stage larva (Fig. 47) is 370–400 μ long and 19–20 μ wide. The oral aperture is central and is connected with the oesophagus by a cylindroid oral capsule 8–9 μ high. The oesophagus is rhabditoid and its length is approximately half that of the body. The nerve ring and the excretory pore are just behind the metacorpus. The anus is 48–56 μ from the tip of the tail. The tail is elongate and straight, without a spine. This stage can survive in water for several months, without undergoing morphological change.

Although an attempt has been made to infect all the intermediate hosts of *Cystocaulus ocreatus* and *Muellerius capillaris* with *P. rufescens*, only the three species *Helicella barbesiana*, *H. vestalis joppensis* and *Monacha syriaca* became infected. Thus the specificity of *Protostrongylus rufescens* to its intermediate hosts is more restricted than that of *Cystocaulus ocreatus* and *Muellerius capillaris*.

The first-stage larva penetrates the tissues of the snail and develops into the second stage very slowly as compared with the larva of *Cystocaulus ocreatus*. The transformation into the second stage (Fig. 48) occurs after 35–38 days at the optimum temperature. The length of the second-stage larva is 600–650 μ, its maximum width is 46–48 μ, and its oral capsule is 7 μ wide. The oesophagus is less than one-third as long as the body. There is an oval excretory gland lying ventrally to the bulbus. The intestine is very distended and dark because its cells are filled with food granules. The anus is 45–50 μ from the tip of the tail. The larva remains at this stage for about 6 days, when the second lethargus commences. After another 6 days, or 46–49 days after the larva has penetrated into the snail, the final, infective stage (Fig. 49) appears, without being preceded by an intermediate pre-infective stage. The infective larva of this species, unlike the infective larvae of all known metastrongylid species, is enclosed in a rigid, brown, spirally coiled, transversely striated, puparium-like casing, which apparently originates in the skin of the first-stage larva. Pressure easily ruptures the casing and liberates the larva (Pl. III, fig. 50), which is devoid of any detached sheaths and is similar to the infective larvae of other metastrongylids. Its length is 620–700 μ, its maximum width 42–44 μ. The oesophagus is about one-third as long as the body. The intestine is narrow and transparent. The anus is 50–52 μ from the tip of the tail. The larva shows only slight movement within the casing, but is very active when liberated in water.

The development just described occurred at 20–30° C. At lower temperatures it is retarded and below 8° C. no development was observed over a period of 5 months. In order to determine whether the retardation due to lower temperatures affects further development, artificially infected snails of three appropriate species were maintained at 4–8° C. immediately after the infecting experiment. Fifty days later, after it was ascertained that no development had occurred, some of the snails were transferred to room temperature, while the rest were left at 4–8° C. In snails kept at room temperature development proceeded at a normal rate, as if the snails had been recently infected, while no development was observed in the refrigerated snails.

As stated above, all but three of the snail species tested resisted infection. The larvae were unable to penetrate into any of the other species, except *Theba pisana*, into which some larvae did penetrate. These, however, did not survive. They were found dead after short periods, while the surrounding snail tissue showed a marked reaction.

A suckling kid was fed the teased feet of *Helicella vestalis joppensis* and *Monacha syriaca* containing several dozen infective larvae of *Protostrongylus rufescens*. The kid died 18 days later of an intercurrent infection. No gross pathological lesions were observed in its lungs, but ten young females of *P. rufescens*, a few mm. long, were found in its small bronchi.

Natural infection of snails by the lung nematodes of sheep

Five hundred specimens of *Helicella barbesiana* were collected between September and October 1945, on a field near Jerusalem, which was heavily polluted with the excrement of sheep and goats. Of these snails 17·2 % were found to be infected with one or more species of the larvae of *Cystocaulus*

ocreatus, *Muellerius capillaris* and *Protostrongylus rufescens*. Larvae of *Cystocaulus ocreatus* were found in 64% of them, larvae of *Muellerius capillaris* in 24% and larvae of *Protostrongylus rufescens* in 22%. Most of the snails were infected with one species only, a few had double infections, while only a single snail had a triple infection. Between 60 and 70% of the snails, depending on the nematode species, harboured only one larva and only in one instance did a snail harbour nine larvae of *Muellerius capillaris*. We have never observed natural infections as intense as artificial infections, in which as many as 200 larvae have been observed in a single snail. In cases of infection with *Cystocaulus ocreatus* all the larvae were in the infective stage. Most of the *Protostrongylus rufescens* larvae were in the infective stage and only a few in the second stage. In most cases of *Muellerius capillaris* infection a mixture of several developmental stages was found. Only first-stage larvae were found in some cases of *M. capillaris* infection and this is taken as an indication that infection at least with *M. capillaris* may occur throughout the season.

During the same period completely dried faecal pellets of sheep were collected from the same field and were examined for the presence of metastrongylid larvae of the species which were found in snails. When the pellets were put into water, they yielded first-stage larvae of all the three species mentioned.

SUMMARY

Five species of lung nematodes of sheep and goats were found in the Levant countries, namely: *Dictyocaulus filaria*, *Cystocaulus ocreatus*, *Muellerius capillaris*, *Protostrongylus rufescens* and *Neostrongylus linearis*. *Cystocaulus ocreatus* is the commonest, while *Neostrongylus linearis* is very rare. Descriptions of the morphology of the adults and of all the developmental stages of all these species, except the last, are presented. The presence of a small but well-developed bursa of a protostrongylid type in *Muellerius capillaris* has been established. The life histories of *Cystocaulus ocreatus*, *Muellerius capillaris* and *Protostrongylus rufescens* have been studied and the susceptibility of various snail hosts has been determined. Experimental infections with larvae of *Cystocaulus ocreatus* and *Muellerius capillaris* have been induced in eleven species of terrestrial snails and slugs (belonging to seven genera). Only three of these eleven species proved susceptible to infection with larvae of *Protostrongylus rufescens*. Experimental infections of definitive hosts with these three species have been obtained. Lowering the temperature retards larval development in the snail. The larval development of *P. rufescens* is especially retarded and its larvae do not develop at all at temperatures lower than 4–8° C. Natural infection of *Helicella barbesiana* with the larvae of *Cystocaulus ocreatus*, *Muellerius capillaris* and *Protostrongylus rufescens* has been found in the vicinity of Jerusalem.

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TWO NEW LUNG NEMATODES FROM NEAR-EAST MAMMALS

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(With 19 Figures in the Text)

In the course of an investigation of the lung nematodes of local mammals the author has established two hitherto unknown species, one from the hedgehog and the other from the mongoose. Descriptions of these species follow:

Crenosoma lophocara n.sp. (Figs. 1–10)

Definitive host. *Erinaceus roumanicus sacer* (found in two host specimens).

Localization. Bronchi.

Geographical distribution. Northern Syria.

The body cuticle is covered with fine, transverse striations, similar to those in many other nematodes. The worm is enveloped by a thin, transparent teguminal sheath which is distinctly wider than the body and is divided by transverse folds into wide rings throughout the almost entire body length. The posterior edge of each of these rings is wider than the anterior edge of the following ring, thus causing a distinct crenation. Each of these rings is provided with fine, longitudinal striation, and the posterior end of each stria is emphasized by a dot, which makes the division of the teguminal sheath into rings very prominent. The first ring appears at the level of the posterior part of the oesophagus, and the most posterior one occurs cephalad to the anus in the female or to the bursa in the male. The crenation is more distinct in the anterior portion of the worm.

It is evident that, in contrast to the prevailing opinion that the crenation of the species of the genus *Crenosoma* is caused by striation of the cuticle, the crenation of *C. lophocara* is caused by striation of the teguminal sheath. It seems likely that this is also true for other species of this genus.

The body is cylindroid and tapers slightly toward both extremities. The oral opening is oval, with the shorter diameter dorso-ventral. Six perityls surround the oral opening, the two lateral ones being smaller than the others. There is a minute sensory papilla at the tip of each perityl. Behind each sublateral perityl are found a pair of papillae closely adhering to each other, while behind each of the lateral perityls there is a very minute latero-ventral papilla in addition to an amphid. Altogether, there are sixteen sensory papillae in addition to two amphids. The anterior extremity of the worm is covered by a cap composed of a granular mass which hinders investigation of the perioral structures. There is no oral capsule, and the oral opening leads to the oesophagus through a small funnel-like aperture. The oesophagus is club-shaped, provided with an indistinct metacorpus. The excretory opening is situated at the level of the middle of the oesophagus, and is connected through a narrow duct with two elongated excretory glands, 0·8–0·9 mm. long. These glands are wider at their anterior ends, and terminate in a small knob. Each gland is provided with a large nucleus. Bottle-shaped deirids are situated at the level of the excretory pore, and their narrow extremities are directed anteriorly.

Male. Length 5·6–6·3 mm., maximum width 0·27–0·31 mm. Oesophagus 0·24–0·31 mm., ratio to the body length approx. 1 : 25. The following is the bursal structure: dorsal ray single, wider than other rays, with a narrow middle portion, its distal extremity covered with a granular layer. It is provided with a small conical papilla on its ventral side. Externo-dorsal rays separate; medio- and postero-laterals fused through their proximal third; externo-laterals, the smallest in the bursa, separate; the ventrals are the longest rays and arise from a wide common base. On the tip of each ray, except the dorsal one, a distinct globular papilla is to be found. Spicules are equal in size and shape, 0·33–0·37 mm. long and of spongy structure. Each spicule is split for two-thirds of its length into two branches, dorsal and ventral. The ventral branch is a little longer and wider than the dorsal one and its tip is slightly bent. The spicules are covered with a thin, transparent sheath. The gubernaculum is a narrow elongated body, 0·11–0·12 mm. long, hook-shaped in profile and provided with two thin alae in its proximal part.

Female. Length 13·3–15·6 mm., maximum width 0·42–0·44 mm. Oesophagus 0·31–0·33 mm. Genital tubules occupy all of the free space in the body. The vulva is situated a little in front to the middle of the body, and projects in the shape of an oval body. Vagina short, sac-shaped. Uteri amphidelph, and there is a sphincter at their junction with the vagina. The anus is situated 0·16–0·20 mm. from the tip of the tail. The tail is conoid, and two subventrally situated phasmids are found just before its tip. Eggs in all stages of development occur in the uteri, including those with fully developed larva. Ova 56–67 × 43–51 μ.

* This study was carried out in the Department of Parasitology, Hebrew University, Jerusalem. The author wishes to express his appreciation to Prof. G. G. Witenberg for helpful advice.

Fig. 1
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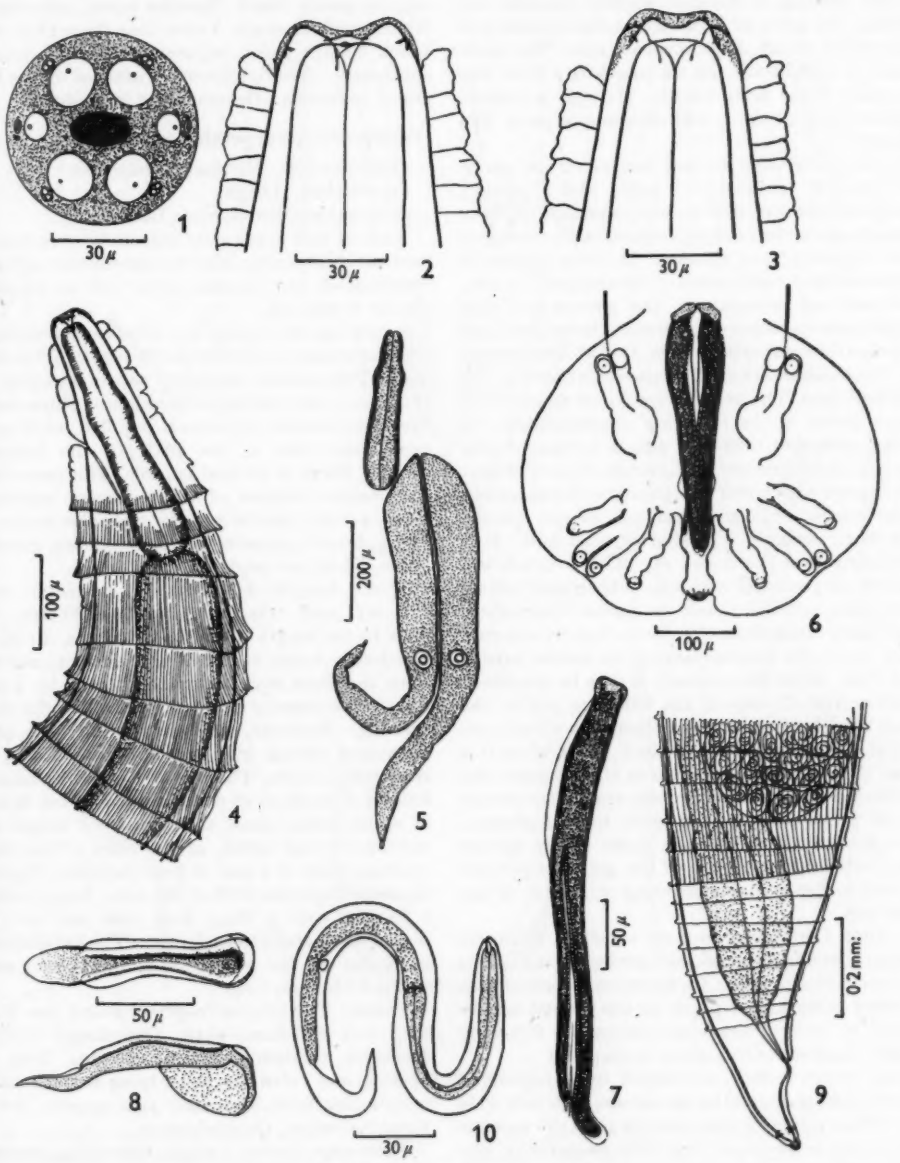

Figs. 1–10. *Crenosoma lophocara.*

Fig. 1. Perioral structures.
Fig. 2. Anterior extremity, lateral view.
Fig. 3. Anterior extremity, ventral view.
Fig. 4. Anterior extremity, low-power magnification.
Fig. 5. Oesophagus and excretory glands.
Fig. 6. Male bursa.
Fig. 7. Spicule.
Fig. 8. Gubernaculum, ventral and lateral view.
Fig. 9. Female, posterior extremity.
Fig. 10. First-stage larva.

The first-stage larva is 270 μ long and 14 μ wide. Its oral opening is directed slightly dorsally (resembling the larva of *Troglostrongylus brevior*) and leads into a small, sclerotized capsule. The oesophagus is rhabditoid, and its length is a little less than half of the body length. The tail is conoid, elongated and straight, and measures approx. 30 μ in length.

Three of the nine known species of the genus *Crenosoma* (*C. striatum*, *C. goblei* and *C. taiga*), closely resemble the new species, especially in their common character: the pigmented, well-developed dorsal branch of the spicules. In other species of this genus the dorsal branch of the spicules is poorly developed and transparent. The author had only a single male specimen of *C. striatum* from *Erinaceus europaeus* for comparison (Figs. 11–13). The evaluation of the characters of other species is based on the published data. The new species differs from *Crenosoma striatum* in the following characteristics: its spicules are larger, 0·33–0·37 mm. as compared with 0·24 mm.; its gubernaculum is larger, 0·11–0·12 mm. as compared with 0·085 mm.; and the dorsal branch of the spicule is longer than half of the spicule, while in *C. striatum* it is shorter than half. It is noteworthy that the dorsal ray of *C. striatum* was found to be provided with two subterminal lateral appendages not mentioned in earlier description. *C. lophocara* differs from *C. goblei* in that the externo-lateral ray is the shortest among the lateral rays in the former, while the contrary is true in the latter. It differs from *C. taiga* in the following points: the dorsal branch of the spicule comprises about one-third the length of the spicule in *C. taiga*, while it is longer than half of the spicule in *C. lophocara*; the externo-lateral ray is the longest among the lateral rays in *C. taiga* and the shortest in *C. lophocara*. *C. lophocara* differs from all three of the species mentioned in the presence of the granular perioral cap and in the granular covering of the tip of the dorsal ray.

In 1945 Dougherty gave an excellent historical summary and review of the species of the genus *Crenosoma* Molin, 1861. Its taxonomic position was discussed in an earlier paper by the present author (1949). In view of new observations the following revised diagnosis of this genus is proposed.

Small stout worms, enveloped by a teguminal sheath which is divided by transverse folds into wide rings whose posterior edge is wider than the anterior edge of the subsequent ring, thus rendering a crenated outline to the worm. Oral opening central, surrounded by six papillae of the internal circle and ten papillae of the external circle, in addition to two amphids. Oesophagus of uniform structure. There is a pair of excretory glands. Deirids present. Male bursa well developed. Dorsal ray single, may be provided with papillar appendages. Postero- and medio-lateral rays are partly fused. Ventral rays may be partly fused. Spicules equal, split distally. Gubernaculum single. Vulva near the middle of the body. Vagina short, separated from the uteri by sphincters. Ovoviviparous. Parasites of the bronchi of mammals. Development in molluscs.

Stenuroides herpestis n.g., n.sp. (Figs. 14–19)

Definitive host. *Herpestes ichneumon.*

Localization. Lungs.

Geographical distribution. Israel.

Each of two mongooses examined were found to harbour this species. Male specimens were extracted undamaged, but females were torn to fragments during extraction.

Adults are enveloped in a wide teguminal sheath bearing numerous folds over the length of the entire body. The anterior extremity tapers abruptly. The triangular oral opening is guarded by three shallow lips. The number of perioral papillae could not be ascertained due to the folds of the teguminal sheath. There is no oral capsule. The pear-shaped oesophagus consists of two separate anatomical parts: a short, narrow anterior muscular region, and a long, broad posterior region containing glandular tissue. There are no deirids.

Male. Length 9·7–11·3 mm., maximum width 0·20–0·21 mm. Oesophagus 0·29–0·30 mm. long; ratio to the length of the body approx. 1 : 35. The copulatory bursa is not of the conventional type, since the short rays are not supported by a membrane. The bursa is situated just behind the cloacal opening. However, the surrounding folds of the teguminal sheath give the appearance of a true strongyloid bursa. The bursal rays are arranged as follows: a single short dorsal ray, preceded by a pair of short lateral rays and a pair of longer rays. Anterior to the latter, at the sides of the cloacal opening, there is a pair of large papillae. The equal, separated spicules 0·08–0·085 mm. long, resemble a feather with a thin, long stem and two alae, placed at an angle to each other. The gubernaculum is single, in the shape of a short rod, and is 0·022–0·024 mm. long.

Female. The longest fragment found was 15 mm. long and 0·72 mm. wide. Oesophagus 0·27 mm. Posterior extremity blunt, carrying both anal opening and vulva, the latter lying at the bottom of a cuticular fold. The uteri join approx. 0·6 mm. from the vulva. Ovoviviparous.

First-stage larva: Length 300–310 μ, maximum width 12–13 μ. Oral opening central, leading to the cylindroid capsule, approx. 10 μ long. The oesophagus, a little less than half as long as the body, has a distinct metacorpus. Excretory pore at the level of the middle of the oesophagus. Length of the tail 40 μ, its tip being straight and smooth.

The above characters place the species in the

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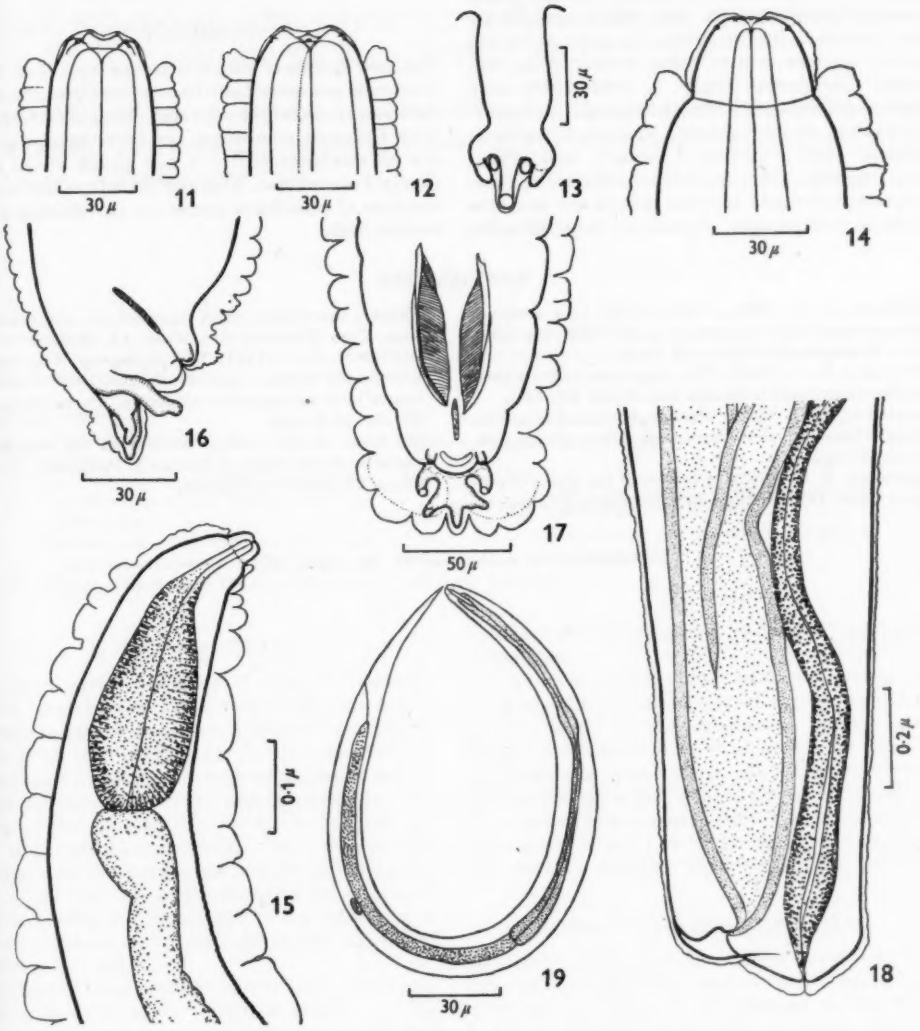

Figs. 11–19.

Figs. 11–13. *Crenosoma striatum.*

Fig. 11. Anterior extremity, ventral view.
Fig. 12. Anterior extremity, lateral view.
Fig. 13. Dorsal ray.

Figs. 14–19. *Stenuroides herpestis.*

Fig. 14. Anterior extremity, ventral view.
Fig. 15. Anterior extremity, low-power magnification.
Fig. 16. Posterior extremity of male, lateral view (spicules omitted).
Fig. 17. Posterior extremity of male, ventral view.
Fig. 18. Posterior extremity of female.
Fig. 19. First-stage larva.

subfamily Pseudaliinae, but it does not fit into any of the known genera, and a new genus, *Stenuroides*, is proposed to include it. The name of the new genus is meant to emphasize its close relationship to the genus *Stenurus*. It differs from the latter in having separate spicules and in being devoid of an oral capsule. Dougherty (1943*a*, *b*, 1944, 1949) fully reviewed the subfamily Pseudaliinae and defined it as composed of four genera: *Halocercus* Baylis & Daubney, 1925, *Pharurus* Leuckart, 1848, *Pseudalius* Dujardin, 1845, and *Stenurus* Dujardin, 1945. All species belonging to these genera are parasites of Cetacea (Odontoceti). *Stenuroides herpestis* is the only known example of a pseudaliin nematode parasitizing a terrestrial host.

SUMMARY

Two new species of lung nematodes from Near-East mammals are described: *Crenosoma lophocara* from *Erinaceus roumanicus sacer* and *Stenuroides herpestis* from *Herpestes ichneumon*, the latter species giving rise to establishment of a new genus of the subfamily Pseudaliinae. This species is the only known example of pseudaliin nematode parasitizing a terrestrial host.

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THE SEASONAL AND DIURNAL ACTIVITIES OF INDIVIDUAL SHEEP TICKS (*IXODES RICINUS* L.)

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(With 6 Figures in the Text)

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1. INTRODUCTION

The annual cycle of activity of sheep tick populations in Scotland (MacLeod, 1939; Campbell, 1950), in Wales (Edwards & Arthur, 1947) and in northern England (Milne, 1945*a*, 1947*a*) has now received considerable attention. Nevertheless, many details of the individual life cycle, and in particular, the timing of the cycle in relation to the seasons, are still imperfectly understood. For interpreting seasonal activity curves we require a knowledge both of the rate of development of the engorged ticks under field conditions and of the behaviour of the unfed stages and their capacity to survive in the absence of hosts.

The first of these requirements is being met in the comprehensive work of Campbell (1950). In the present paper we have attempted to approach the second question—the seasonal activity and persistence of individual unfed ticks—by studying the behaviour of some experimental populations. These have consisted of unfed ticks reared under controlled conditions, and therefore of known age, which were placed at different seasons in a habitat natural to this species. The characteristic movements are so restricted that it was found possible to follow the day-to-day activities of marked individuals without difficulty. As hosts could be excluded from the habitat by suitable fences and 'cages', a general picture of the entire activity of the individual could be built up.

In a previous study of the senses of the sheep tick, a variety of perceptions and orientations were demonstrated in the laboratory (Lees, 1948). Certain inferences were then drawn as to their significance in host-finding. The present observations on the behaviour of active ticks in the natural environment have served both to test and to amplify these conclusions. The behaviour of the engorged tick has been described elsewhere (Milne, 1950*a*).

2. METHODS AND MATERIAL

As is well known, the whole life of the sheep tick is spent in the vegetation, with the exception of three brief periods when it is attached to its hosts. Engorged, moulting and quiescent unfed ticks are all found at the vegetation base in the 'mat' of decomposing plant material (Milne, 1950*a*). On becoming active the tick climbs upwards to the vegetation tips where it comes to rest. Our object was to place unfed ticks of known age at the vegetation base and to record their subsequent activity at the tips. This type of experiment is made possible by the limited extent of the lateral movements (Milne, 1950*a*). Active ticks, following the upright stems of the vegetation, tend to move almost entirely in a vertical direction (p. 14).

By 'activity' we mean the presence of the tick at the vegetation tips. It is shown below that when in this position the tick is usually in a 'mood' for responding to 'host stimuli', whereas 'inactive' individuals at the vegetation base are not. As the ecologist assesses activity (Milne, 1943) either by counting engorging ticks on the host or by blanket dragging (the latter depending on the willingness of ticks at the vegetation tips to cling to any moving object), all three definitions are approximately coincident. It may be noted that in the present context the term 'activity' does not necessarily denote that the tick is moving on the vegetation; indeed such 'active' ticks are usually at rest (see p. 199). In practice, the assessment of activity by a simple daily observation of position proved satisfactory.

Each tick received one or two spots in four colours; twenty-four colour combinations were therefore recognizable, and this number of ticks of each sex was usually laid down at each station. Active adults on the long stems of the vegetation clump were readily visible; at each observation the markings of active adults were noted and they were then left undisturbed. Any ticks that were not visible were regarded as 'inactive'.

As nymphs and larvae proved to be too small for marking individually, a second sampling method was adopted. This involved brushing the vegetation with the hands. Any active ticks transferred themselves with alacrity and were then counted and destroyed. The procedure was for the observer to brush and comb through the vegetation with the fingers twenty times or until such time as no more

Table 1

Lot	Station nos.	Date laid down	Material	Date of emergence	Recoveries Nos.	Recoveries %
1	1–3	7 May	24 ♂♂, 24 ♀♀ each	20 Mar.	23, 38	32, 53
	4	7 May	20 ♂♂	20 Mar.	6	30
	5	7 May	400 nymphs	15 Mar.	234	59
	6	7 May	Larvae, progeny of 1 ♀	25 Mar.	931	—
2	7	27 July	24 ♂♂, 24 ♀♀	25 June	17, 13	71, 54
	8	27 July	100 nymphs	15 June	29	29
	9	27 July	Larvae, progeny of 2 ♀♀	10 June	1451	—
3	10, 11, 12 & 13	12 Oct.	24 ♂♂, 24 ♀♀ each	5 Aug.	56, 54	59, 56
	14	12 Oct.	400 nymphs	1 Sept.	129	33
	15	12 Oct.	Larvae, progeny of 1♀	10 Sept.	1133	—
	16	12 Oct.	Larvae, progeny of 1♀	10 Sept.	173	—
		Total	212 ♂♂		102	48
			192 ♀♀		105	55
			900 nymphs		392	44

The situation selected for the experiments was a naturally infested hillside at Hethpool, northern Northumberland. An area 20 yards square was fenced so as to exclude large hosts, particularly sheep. Within this enclosure a number of stations were prepared, each consisting of a clump of vegetation with intermingled rough grasses and rushes. In order to prevent birds from carrying away active ticks, a cylindrical 'cage', 2 ft. high and 2 ft. in diameter, and constructed of ½ in. wire-netting stretched on a wire frame, was inverted over each site. The clumps were all 1 ft. in diameter and the vegetation round the periphery was kept closely cropped to a distance of 6 in. outside the cage. The longer stems in each were periodically shortened to prevent them from touching the top of the cage.

Two methods were used for sampling the clumps. Adults were sufficiently large to be marked individually on the dorsum with spots of cellulose paint. ticks appeared on his hands. This method, which yields data only on the time of onset of activity, is obviously less informative than the method of direct observation employed with adults.

Some details concerning the material laid down at the sixteen separate stations, together with the totals recovered, are given in Table 1. The age of the ticks in each batch was determined from the date when a rough estimate showed that about half the ticks had moulted; the usual age was 4–6 weeks at the time of laying down, that is, all were 'young' ticks.

Three lots were laid down during 1945, on 7 May, 27 July and 12 October respectively. Those in lots 1 and 2 had been reared on hedgehogs and had subsequently moulted in the laboratory. Although these batches were carefully acclimatized to the lower outdoor temperatures before being set out, it might be contended that the high incubation

temperature (24° C.) to which the engorged ticks were subjected would affect their activity subsequently. This was not considered probable, for Mellanby (1939) has demonstrated that in a number of insects thermal adaptation, as judged by activity, always takes place rapidly, usually within 20 hr. The sheep tick shows similar adaptive changes in the chill-coma temperature. If the rate of acclimatization is also comparable, any after-effects of exposure to high developmental temperatures would not be expected to persist for long in the lives of the succeeding unfed stages. However, in order to test this point specifically, the ticks in lot 3 were taken from two sources. Those placed at stations 10 and 11 were reared in the laboratory as described above; those at stations 12 and 13 were derived from engorged ticks collected from sheep at Hethpool during the normal spring activity period. The latter were stored during the summer in a cool outhouse where they moulted. In spite of their different origins, no differences in the activity of laboratory-reared and 'wild' ticks were detected subsequently.

The ticks were transferred from the culture tubes to their position in the mat at the base of the clumps with as little disturbance as possible. Yet it is probable that this treatment sometimes stimulated larvae and nymphs to premature activity (see p. 205). With adults this error cannot be serious, as they invariably remained quiescent for some weeks at least before becoming active.

The observations, which began on 8 May 1945 were continued at intervals until 29 July 1947—about 10 months after the last active tick was seen. During these months 415 separate day observations were carried out; and on 43 days two observations were made. During periods of activity daily observations were attempted. Nevertheless, even during the peak of seasonal activity, the site could seldom be visited on more than 6 days in the week. In compiling the activity records of adults, therefore, the following convention has been adopted: individuals were counted as active on the day when no visit was made if they were at the vegetation tips on both the preceding and succeeding days; and as inactive if absent from the tips on either day. Experience showed that the number of observations could safely be reduced to one per week or even one per fortnight during the winter months when all activity was virtually at a standstill.

3. SEASONAL ACTIVITY

(i) *Larvae and nymphs*

Recently emerged larvae and nymphs were laid down in May, July and October 1945 at the stations indicated in Table 1. The numbers recovered by the sampling method described above are given in Table 2 as the monthly totals. The frequency of sampling was sufficient to ensure that any active ticks would very quickly be removed from the vegetation.

Larvae, even those in the October series, became active with little loss of time. Indeed some were always recovered the day after the clusters were laid down in the vegetation and in each case the majority were recovered over the next 2 months. In the May and July series only a few remained for recovery during April-June 1946 (1 and 3% respectively). In the October series, on the other hand, a substantial proportion (24%) overwintered in a quiescent condition, appearing on the vegetation during 1946.

Larvae at stations 16 and 15 were laid down on the same day, but the former were left undisturbed until 11 March 1946 when sampling was begun. During October and November dense clusters had been observed near the tips of some of the taller rushes at this station; but the active larvae composing these clusters gradually dispersed and disappeared as winter approached. Next April–June the recoveries at this station were no more numerous than from station 15, although the numbers laid down initially were about the same (one larval cluster per cage). This is some indication that larvae

Table 2. *Seasonal activity of nymphs and larvae laid down during* 1945

(The numbers recovered are given as monthly totals.)

	1945								1946							
Station	M.	J.	J.	A.	S.	O.	N.	D.	J.	F.	M.	A.	M.	J.	J.	A.
								Larvae								
6	548	118	90	5	4	0	0	0	0	0	0	1	5	2	0	0
9	—	—	482	897	19	1	0	0	0	0	0	0	35	2	0	0
15	—	—	—	—	—	649	30	0	0	0	0	9	129	66	11	0
16	—	—	—	—	—	—	—	—	—	—	1	12	123	34	3	0
								Nymphs								
5	24	66	107	30	4	0	0	0	0	0	0	1	0	1	1	0
8	—	—	—	13	0	1	0	0	0	0	0	4	10	1	0	0
14	—	—	—	—	—	18	12	0	0	0	0	43	51	3	2	0

which become active in October–November may fail to survive into the following season. Detailed information on this topic is available for adults (see below).

The onset of nymphal activity was somewhat more delayed, although in the May series 64 % of the total recoveries were made over the following 3 months. Comparing their activity with larvae, a higher proportion from the July and October series (namely, 52 and 81 % respectively) remained quiescent until the following spring (Table 2).

No larvae or nymphs were recovered from these stations after July 1946.

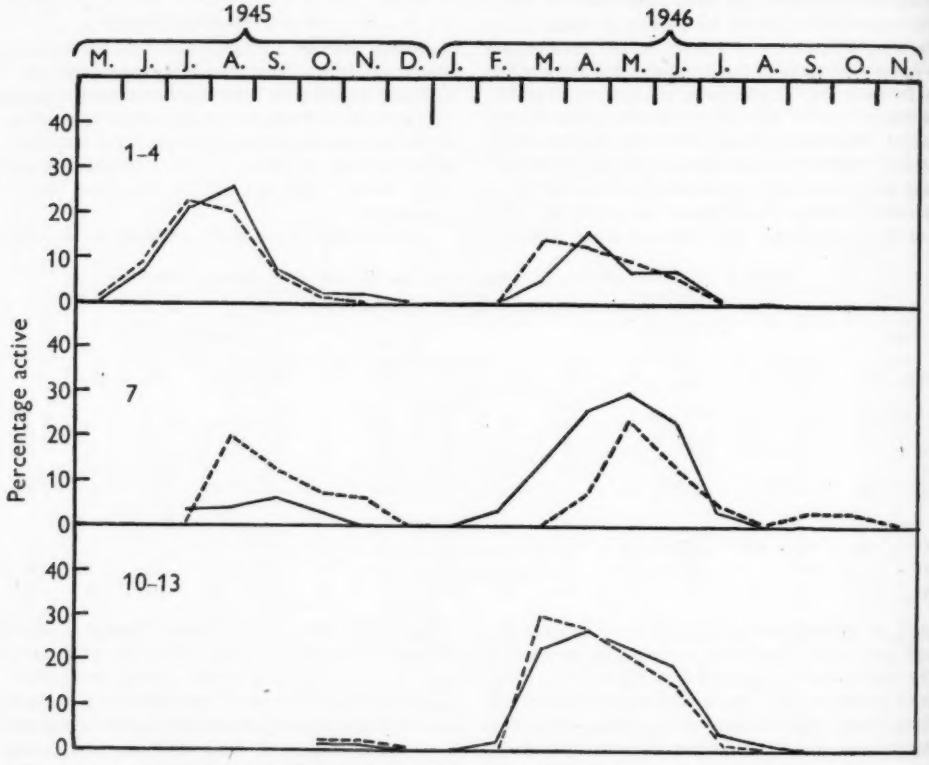

Fig. 1. Seasonal activity of adult ticks at stations 1–13. Continuous lines, males; broken lines, females.

(ii) *Adults*

During May, July and October 1945, 212 male and 192 female ticks with individual markings were laid down at the stations indicated in Table 1. Of these, 48 % of the males and 55 % of the females were seen again on the vegetation. The last adult tick was observed on 3 October 1946.

A general picture of the seasonal activity of the whole adult population is given by summing the numbers of different individuals that appeared each month. In Fig. 1 these monthly totals are expressed as percentages of the total monthly records over the whole period of activity.

As already mentioned, newly moulted adults tend to remain quiescent for longer periods than either larvae or nymphs and usually at least 1 month passed before the first ticks appeared on the vegetation. In the series set out in May, considerable activity developed during July–August 1945 but died away during the winter months; activity then recrudesced during the spring of 1946. Ticks in the July series behaved similarly, save that a larger proportion of the 'total activity' occurred in 1946. In the October series the prevailing low temperatures prevented all activity (except in one individual) until the following spring.

(iii) *Seasonal activity of individual adults*

The composition of the activity peaks in the adult population is revealed by an examination of the individual records of the 207 ticks subsequently seen on the vegetation. The active life of these ticks

was distributed over 1–6 calendar months which were sometimes, but not always, consecutive. For simplicity of presentation all the different monthly combinations are set out in Fig. 2, together with the numbers of ticks in the three series which appeared during each month-combination. Activity during any one month does not of course imply that such activity was continuous—it may have occurred on only one day. The behaviour of male and female ticks was so similar that their records are not shown separately in this chart.

The study of Fig. 2 immediately demonstrates a feature of the activity behaviour that could not have been foreseen in the absence of individual identifications. Of the sixty-seven ticks in cages 1-4 (May series) thirty-five were active exclusively within the period May–November 1945; twenty-three were active exclusively from March to June 1946; only nine were active during both periods. That is, the majority of individuals active during summer did not appear the following spring when the active population consisted in the main of ticks that had remained quiescent for approximately 12 months. The arrangement followed in Fig. 2 is intended to bring out this point.

The same feature can be observed in the behaviour of the July series. Six ticks out of thirty still went through their entire activity within the period July–November 1945. Twenty-one became active for the first time from March 1946 onwards. Only three were active during both periods.

The active life of 110 ticks laid down in October 1945 fell, with the one exception already mentioned, within the period February–October 1946. None appeared again in the spring of 1947.

(iv) *The duration of the active period in adult ticks*

The results summarized in the previous section show that some adult ticks in the experimental populations remain quiescent for many months before becoming active for the first time. The *active period*, which may be defined as the interval between the first and last appearances on the vegetation, is often comparatively short. In the few ticks exhibiting both summer (or autumn) and spring activity, the active period is, of course, extended by the low temperatures of winter. For example, the activity of thirteen individuals behaving in this way was spread over an average period of 247 days. If these be omitted there remain 194 ticks whose active lives fell entirely within 1 calendar year. Among these the mean duration of the active period was 29·5 days in males, and 31·7 days in females. However, the limits of variation were very wide (1–123 days).

The time actually spent by the tick at the vegetation tips is normally considerably shorter than the duration of the whole active life. The number of days thus spent is given in Table 3. Among 207 ticks, males were at the tips for an average of 7·3 days and females for 10·9 days. The minimum activity was 1 day, the maximum (in a female) 54 days.

Reference to Table 3 shows that many of the ticks (24 %) appeared on 1 day only. Some of these may have died prematurely either from the attacks of predators (unhindered by the cage, e.g. shrews and arthropods), or from experiencing unsuitable microclimatic conditions (Milne, 1950*a*). Very few, if any, would have moved outside the vegetation clump (Milne, 1950*a*). Although such explanations probably account for some of these records, they may not necessarily account for all. There can be no doubt, as the following examples show, that some individuals appear very infrequently and achieve only a few days of activity.

Table 3. *The time spent by* 207 *adult ticks in activity at the vegetation tips*

	Days of activity																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19
♂♂	31	13	9	7	3	3	1	0	4	2	3	0	3	3	2	1	2	1
♀♀	18	13	8	9	5	1	6	2	6	1	1	1	1	1	2	3	3	0

	Days of activity																	
	20	21	22	23	24	25	26	27	28	29	31	32	33	34	38	39	40	54
♂♂	4	0	1	1	2	1	3	0	1	0	0	1	0	0	0	0	0	0
♀♀	1	2	3	2	0	2	0	1	2	1	1	1	1	2	1	1	2	1

(*a*) Male W/M from station 2 was seen for 1 day, 381 days after it was placed in the vegetation. (*b*) Male –/L at station 11 was active for 1 day, disappeared for 121 days, then reappeared for 1 day. (*c*) Female M/W at station 1 was at the tips for 1 day, and was then, successively, quiescent for 60 days, active for 1 day, quiescent for 254 days, active for 1 day. In such instances where it is reasonable to suppose that we have indeed observed the whole of the activity, the short active life would appear to be due to individual sluggishness.

While considering the duration of activity at the tips a further statement may be added regarding the behaviour of ticks that appeared during 1 year exclusively or during 2 (that is, in both summer or autumn and spring). In the May series (stations 1–4) ticks that were active only in 1945 spent an average of 9·3 days at the tips; those appearing only in 1946

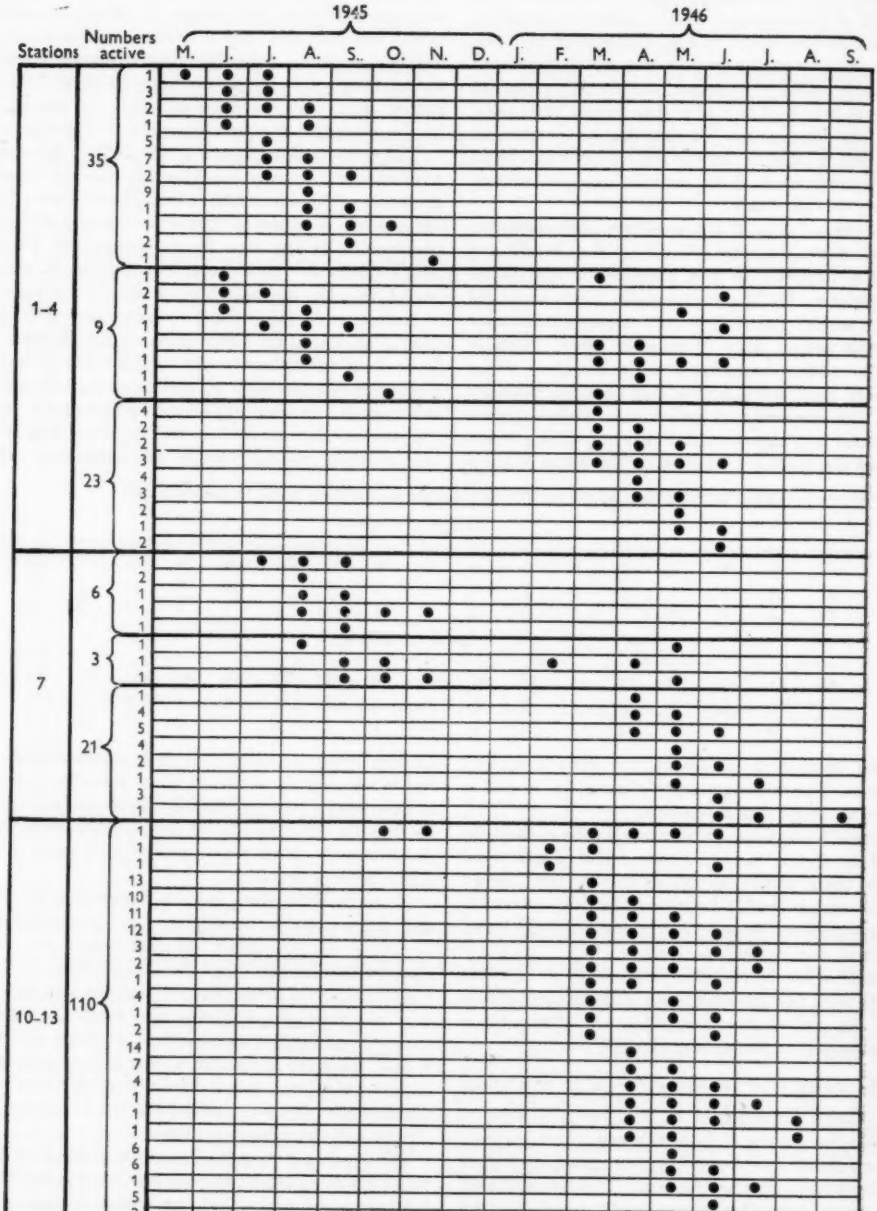

Fig. 2. Seasonal activity of individual adult ticks.

spent 9·6 days; and those appearing during both seasons 12·7 days. However, on examining the distribution of the active days in 'two-season' ticks it is found either that the total duration of activity is small or that the activity is distributed very unequally between the 2 years. For example, five ticks spent an average of only 4·2 days at the tips. The remaining four individuals which spent an average of 23·2 days at the tips belong to the second category; the following are examples: (*a*) male M/O was active for 15 days during June and July 1945 and for 1 day during June 1946; (*b*) male –/L was at the tips for 2 days during August 1945 and for 17 days during April and May 1946. Clearly, a long period of activity during the summer greatly reduces the chances of a further long spell of activity during the following spring.

base. Table 5 records the number of separate occasions on which marked ticks in all cages were observed at the tips.

Although a considerable proportion (35 %) made only one appearance, this is largely a reflexion of the numbers that were seen on only 1 day. Among others that appeared only once, the maximum duration of the activity period was 11 days. On the other hand, 65 % appeared on two or more separate occasions. The extreme example was a female that went through as many as nineteen phases of activity at the tips, with, of course, eighteen intervening phases of quiescence (Table 5). The behaviour of both sexes was alike. The average number of active phases in all ticks was 3·6.

The duration of the activity phases was highly variable, as a representative selection of the indivi-

Table 4. *Mean duration of activity in adults in relation to the estimated length of survival*

	Stations 1–4				Station 7					
	Active 1945 only		Active 1946 or 1945–6		Active 1945 only		Active 1946 or 1945–6		Stations 10–13	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Minimum survival (days)	90	94	353	358	53	52	303	321	263	215
Days active	4·1	14·4	8·4	10·7	13·0	12·4	9·1	6·8	7·2	10·2
Percentage	5	15	2	3	24	24	3	2	3	5

Table 5. *The number of separate occasions (activity phases) on which 207 adult ticks appeared at the vegetation tips*

	Number of appearances																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
102 ♂♂	40	22	8	6	7	3	3	6	2	1	3	0	1	0	0	0	0	0	0
105 ♀♀	31	18	13	13	6	3	6	3	4	2	1	2	0	1	0	1	0	0	1

A comparison of the duration of activity with the estimated length of life may also be of interest. Although the time of death is not known precisely, such an estimate may perhaps be based on the assumption that the ticks survive no longer than the day on which they were last seen active. The values obtained have again been grouped according to whether the active period fell within 1 or 2 calendar years (Table 4). When activity is greatly delayed, the time spent at the vegetation tips may amount to no more than 2–3 % of the whole life span.

(v) *Intermittent activity—active and quiescent phases*

That the duration of activity is so much shorter than the average 'active period' is of course accounted for by the fact that adults showing extended activity seldom remain continuously at the vegetation tips. The periods at the tips, which may be termed the phases of activity, are separated by intervals of varying length (inactive or quiescent phases) when the tick is out of sight at the vegetation

dual records shows (Fig. 3). The mean duration was 2·3 days for males and 2·8 days for females. The shortest and longest phases of continuous activity at the tips, in individuals that appeared on more than one occasion, occupied 1 day and 24 days respectively.

The successive active phases of individuals often exhibit little regularity. Their duration may be either approximately equal or highly unequal. This is illustrated in the following six individual records in which the duration of consecutive active phases is given in days:

(*a*) 2, 1, 3, 1, 1, 2, 4, 2, 5
(*b*) 3, 1, 3, 1, 2, 6, 2, 1, 4, 1, 2
(*c*) 4, 2, 3
(*d*) 13, 2, 3, 2, 1, 1, 1
(*e*) 2, 1, 1, 2, 9
(*f*) 1, 2, 17, 3, 1

The quiescent phases are usually longer. If we omit those ticks that were active in two seasons (for here quiescence is influenced by winter temperature

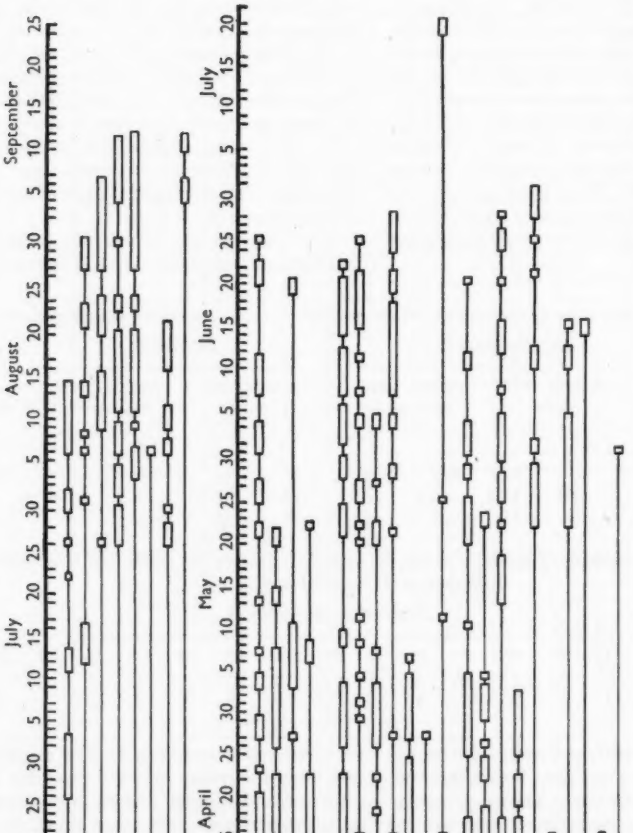

Fig. 3. A representative series of activity records of individual ticks. In each example the whole of the activity has been included, but in the case of spring-active ticks from stations 1–4 and 10–13 the long initial period of quiescence has been omitted. Quiescence is indicated by a line, activity by a rectangle. The observation days are marked by downward strokes along the date lines.

conditions), as well as those exhibiting only one phase of activity, the mean duration of the inactive phases in the remaining 125 ticks was found to be: males, 10·9 days; females, 11·2 days. When the records of these ticks are arranged in two groups containing individuals that were active for more or less than an arbitrary total of 10 days, the average durations of the quiescent phases in the two groups were found to be 7 and 22 days respectively. The means differ significantly ($P = < 0{\cdot}01$). In other words, individuals displaying little activity or 'drive' also remain quiescent for long and irregular periods. In the following extreme examples the inactive phases are denoted by italics:

(*g*) 3, *15*, 1, *70*, 1
(*h*) 1, *43*, 3, *33*, 2, *27*, 1
(*i*) 1, *123*, 1

The most interesting records are provided by very active ticks which, as we have noted, tend to have shorter periods of quiescence. In such cases there is frequently a fairly regular periodicity in the behaviour, relatively long phases of activity at the tips alternating with phases of inactivity, often no longer than 1 day in duration. Several examples are shown in Fig. 3. The following are some additional cases:

(*j*) 3, *1*, 9, *1*, 5, *1*, 4, *1*, 2
(*k*) 3, *8*, 9, *5*, 1, *9*, 6, *1*, 2, *1*, 4, *5*, 2, *7*, 1
(*l*) 9, *9*, 8, *3*, 9, *3*, 2, *10*, *5*, *1*, 3, *1*, *5*, *1*, *5*, *1*, *7*, *1*, 1

A tentative interpretation of intermittent activity of this type is given in the Discussion (p. 207).

(vi) *Activity of adults in the October series*

The treatment of the material composing the October series and the timing of the activity cycle of this population in relation to the seasons, leave little doubt that the pattern of activity will be a reasonably faithful representation of events in the natural 'wild' population at Hethpool. It may therefore be useful to consider this series briefly in isolation from the remaining data.

In this group of 110 ticks the average active period was 35·4 days (males, 36·4 days; females, 34·5 days). As may be deduced from Fig. 2, the percentage of ticks whose activity fell entirely within 1 calendar month was 35%; and the percentages falling within 2, 3, 4 and 5 consecutive months were 24, 18, 16, and 7% respectively. The duration of the active life varied from 1 to 123 days. The total number of days spent at the tips was 7·7 for males (range 1–32) and 10·2 for females (range 1–54). This was made up as follows: on the average males made 3·7 (range 1–13) separate appearances at the tips each lasting for 2·2 days (range 1–13); females made 4·1 (range 1–19) appearances, each lasting 2·5 days (range 1–10).

This may be summarized as follows. In a population showing normal spring activity the adult, if hosts are not available, spends an average of 8–10 days at the tips comprising three to four active phases of 2–3 days each; and the active life encompasses an average period of 5 weeks. This amends the less accurate statement that has appeared in Milne (1950*a*, *b*).

4. DIURNAL ACTIVITY

At times when daytime activity was fairly steady or rising from day to day, Milne (1947*a*) showed that although active nymphs were certainly present at the vegetation tips during the night, nevertheless smaller numbers were collected on the blanket than during the day; and this seemed to be correlated with atmospheric temperature (Milne, 1947*a*, fig. 3). His observation may indicate either that fewer individuals are at the tips at night or that those present are less inclined to cling to the blanket, or both.

Some additional evidence has been gathered by observing the behaviour of individual adult ticks over about 24 hr. For the starting-point, a day in spring (15 April 1946) was selected when a large number of marked adults were active at stations 1–4, 7 and 10–13. Observations were made at 13.30, 15.00, 17.00, 19.00, 21.00, 00.00, 03.00, 05.30, 09.00 and 14.00 hr. G.M.T. from 15 to 16 April. (In addition, the total of active ticks, forty-six, was noted at 08.00 hr. on the 15th.) Sunset was at 19.09 and sunrise at 04.49 hr. G.M.T. Weather conditions were practically stable on the 15th and 16th (maximum temperatures 20 and 18° C. respectively) as the following data indicate:

15 *April, afternoon:* cloud 0/10; sun strong to moderate; warm; no rain; vegetation dry; wind B 2–3.

16 *April, forenoon:* cloud 1/10; sun strong; warm; no rain; vegetation dry; wind B 1–3.

Intervening night: no change except dewfall and normal drop in temperature.

Fifty-nine individual ticks, twenty-eight being females and thirty-one males, were observed. Fig. 4 shows the times at which they were seen active.* Within the observation period, part (or the whole) of one activity phase was exhibited by fifty-three ticks and of two phases by six ticks. Forty-eight of the ticks were active at 13.30 hr. on the 15th and the remaining eleven became active by 19.00 hr. (i.e. before sunset), no *new* ticks appearing thereafter.

Fig. 4 suggests that males and females behaved sufficiently alike for their numbers to be combined. Of the fifty-nine ticks, eleven ceased activity altogether before sunset (group C); fourteen ceased activity during the night, only one reappearing—at 14.00 hr. on 16 April (group B); and thirty-four

* It is important to note that unless otherwise stated in this section 'active' means 'at the vegetation tips' and 'activity' has the same connotation.

continued activity into the next day, two of them with short breaks around 17.00 and 19.00 hr. respectively (group A). There were twelve individual ascents to the active position after 13.30 hr. and before sunset on 15 April and four after sunrise on the 16th, i.e. sixteen ticks became active in daylight and only one during the night (at 21.00 hr.). Thus the individual tick can remain active throughout the night as well as day, and can come up or go down either by day or by night. On the latter point, the above data suggest that the proportion going down was greater by night than by day, but this will be referred to in further detail below.

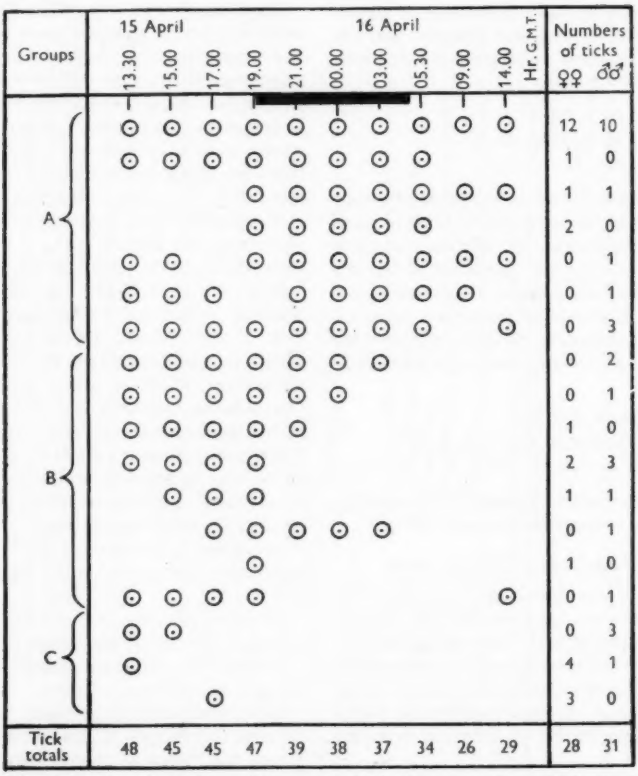

Fig. 4. Numbers of active ticks and times at which they were observed in the 'active' position, i.e. at the vegetation tips.

In Fig. 5 the total active population on all stations at each observation has been plotted together with the hourly atmospheric temperatures. Population during daylight on 15 April (forty-five to forty-eight ticks) was much higher than that during daylight on 16 April (twenty-six to twenty-nine). But the striking fact is that most of the fall in population took place during the night, and particularly (c. 80%) when temperatures were below 10° C., from 20.00 hr. on the 15th to about 07.00 hr. on the 16th (cf. sundown 19.09–04.49). The latter might be called the 'night-temperature period' since atmospheric temperature lags behind the sun.

During the period of study, twenty-two ticks were active at all times (see Fig. 4). Among the remaining thirty-seven ticks there was a total of fifty-three ascents and descents to and from the active position (activity at 13.30 hr. being scored 0). Analysing for the night-temperature period, the tabulation is:

	Ascents	Descents
Above 10° C. (15 April)	12	15
Below 10° C.	1	20
Above 10° C. (16 April)	4	1

This shows that while in the daytime the ascents tended to balance the descents (16 : 16), at night the descents outnumbered the ascents very substantially (20 : 1). With this 2×2 grouping and using Yates's

correction for continuity, χ^2 is 9·93 and P is highly significant ($< 0{\cdot}01$).

It seems fairly certain then that Milne collected fewer ticks on the blanket at night, *mainly* because there are fewer ticks at the tips then, i.e. because the proportion ending an activity phase by night greatly exceeds that by day. A new point emerging is that when day-to-day activity is steady or rising (Milne's data, fig. 3, 1947*a*), the number active at night will be less than on either the preceding or succeeding day; on the other hand, when day-to-day activity is falling rapidly, as in the present data, viz.

April	13	14	15	16	17	18	19
Active ticks	28	—	48	29	19	17	13

the number active at night will be less than that of the preceding day only.

Since there is a drop in activity during the night, then whether day-to-day activity increases, remains steady, or decreases, must depend on the relative numbers of ticks beginning and ending an activity phase during the day. The present data show that when the latter ratio is about unity then day-to-day activity decreases.

5. MOVEMENTS OF TICKS IN THE VEGETATION LAYER

(i) *Orientations*

Ticks on the upper part of the vegetation are usually found to be at rest. Among adults (1752 observations), 87 % were stationary with the forelegs folded or semi-folded, 9 % were questing with both forelegs extended stiffly, only 4 % were walking on the vegetation. The types of orienting movements displayed in the natural environment could be judged by following the movements of the latter. The behaviour has already been investigated in the laboratory, using glass rods as models of the rush and grass stems which the sheep tick is usually seen climbing (Lees, 1948).

The most frequent and important type of orientation observed was a gravity response involving both taxis and kinesis components. After descending a short distance from the tip of the stem the tick turns upward, often repeatedly, and finally comes to rest just below the tip. It may then remain at rest for a period varying from a few minutes to many days. The tracks of three female ticks climbing long *Juncus* stems illustrate this reaction (Fig. 6 A–C). In A the

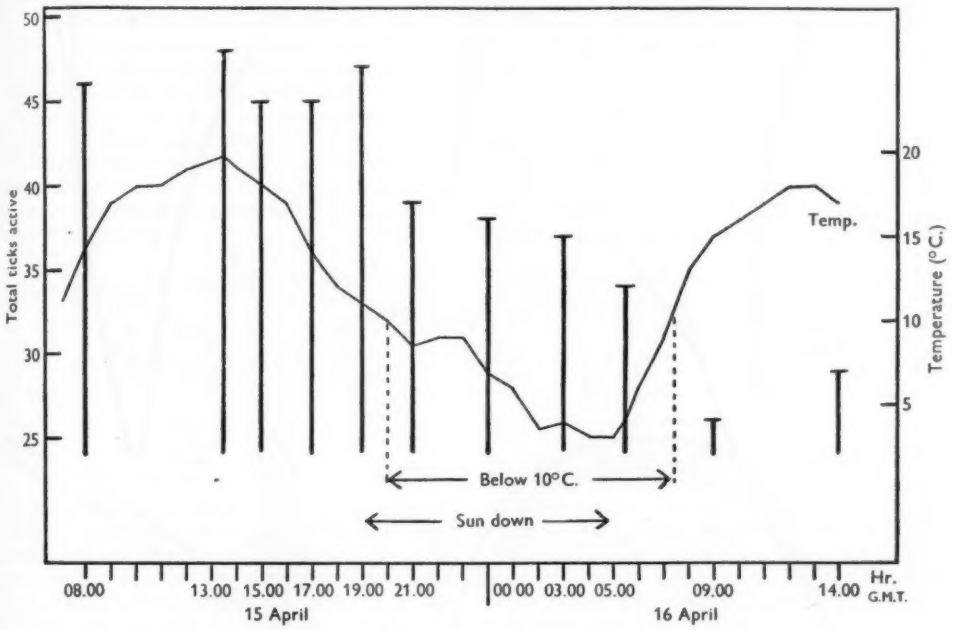

Fig. 5. Total numbers of active ticks at stations 1–4, 7 and 10–13, and atmospheric temperature in nearby screen.

tick climbed from near the base to the tip, started to descend but turned upwards again 2 cm. from the tip; this manoeuvre was then repeated three times before the tick came to rest $\frac{1}{2}$ cm. from the tip with turning' was a response to gravity and not merely a tactile response to the 'tip' of some free object, concerned the behaviour on a curved glass rod (Lees, 1948). The same turning movements occur as

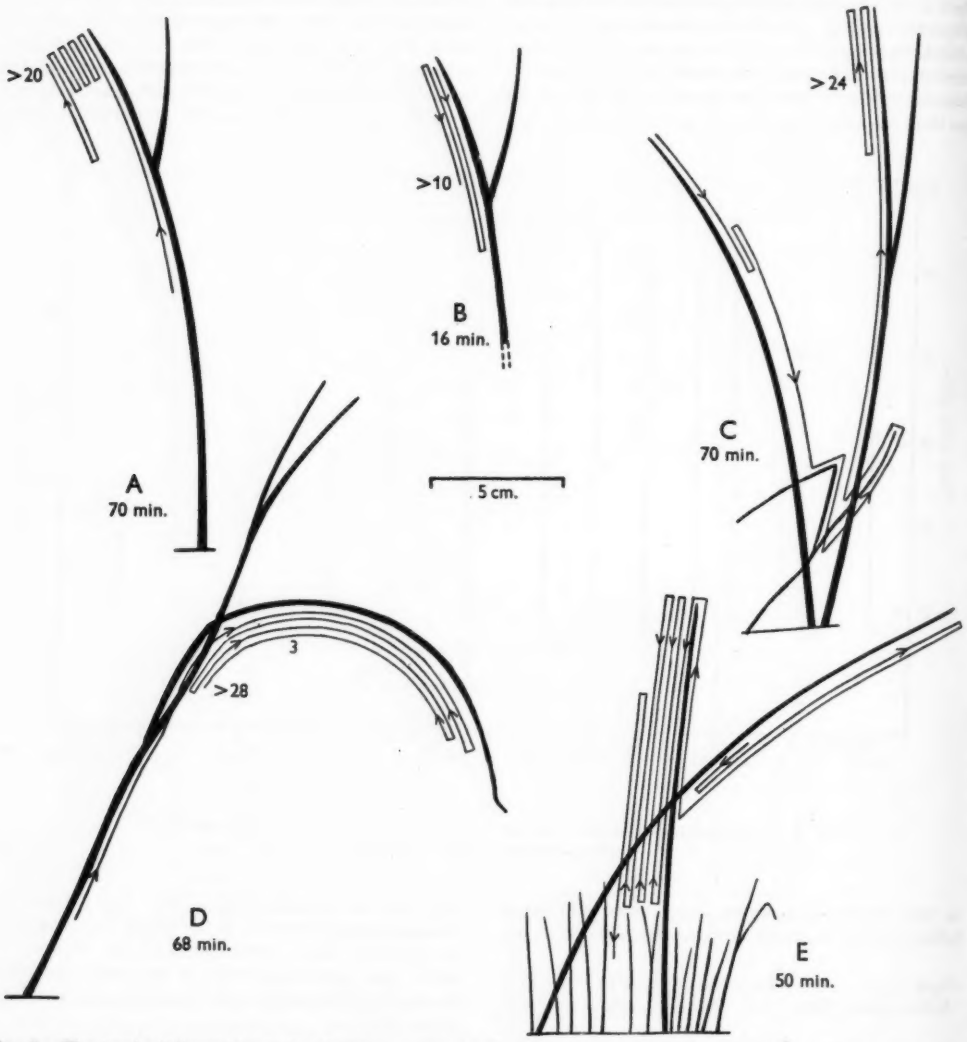

Fig. 6. The tracks followed by ticks climbing on vegetation. The heavier lines show the form of the supporting rush and grass stems. The figures near the base indicate the time, in minutes, taken for the whole manoeuvre; periods spent at rest on the vegetation are also noted at the appropriate points on each track.

the capitulum uppermost. This reaction is also shown in Fig. 6 B (the same stem) and in C, but in the latter example the tick has repeatedly crossed from stem to stem before settling down in the final position of rest.

One experiment which suggested that 'upward the tick begins to walk downwards, even though the free tip has not been reached. A similar situation, in which a male tick was climbing on a rush stem that chanced to be bent into the shape of an arc, was noticed in the field (Fig. 6D). The tick turned

repeatedly at each side of the arc and finally came to rest not far distant from the highest point on the stem.

An orientation of a much more infrequent type is shown in Fig. 6E. This tick (a nymph) apparently did not respond to gravity, for after reaching the tip it continued downwards for some distance. However, it turned upwards at the point where the long stem on which it was climbing passed among a cluster of shorter grass stems; and upward-turning was repeated twice at the same point before the tick finally disappeared into the short grass. This may have been a reaction to humidity for undesiccated ticks usually avoid moist air strongly; and the point of turning corresponded approximately with the steepest part of the humidity gradient which extends vertically from the mat to the vegetation tips (Lees, 1948). Very slight desiccation is sufficient to abolish this reaction; the ultimate failure of the orientation in this instance could have been caused by water losses sustained during the movements in the upper vegetation.

On the whole, however, a remarkably high proportion was found on the tallest stems. In 384 cases where this information was noted, 82 % of the adults were at rest on the tallest stems in the clump. This conveys the impression that the tick is seeking to climb to the highest point available. This appearance may be deceptive for the following reason. At nearly all the stations the tallest stems, usually those of rushes, were also the stoutest, and as such may have been preferred to thin wiry grass stems or flexible blades of grass. In one instance a female tick was observed struggling up a 10 cm. upright grass blade. Apparently finding this support uncomfortable, she soon descended and crossed to a stouter, more rigid stem.

The side of the stem occupied at the final position of rest is largely determined by the direction of the sun and wind. The tick tends to avoid both by sheltering behind the stem.

In 288 observations on adults, seventy (24 %) were classified as 'in sun', eighty (28 %) as 'intermediate' and 138 (48 %) as 'out of sun'. If the ticks had been randomly distributed in the four 90° arcs of the stem circumference, their numbers would have approximated to the ratio 1 : 2 : 1. The departure from this ratio is highly significant ($P = < 0{\cdot}01$).*

Table 6. *The distribution of ticks at three different levels in the vegetation*

Stage	Total	Top third (%)	Middle third (%)	Bottom third (%)
Larvae	3449	94·5	5·4	0·1
Nymphs	392	93·9	5·6	0·5
♀♀	525	72	26	2
♂♂	336	91	8	1

(ii) *The final position in the vegetation*

The significance of these orienting movements (and the gravity response in particular) in bringing the tick to a position of vantage near the tips, could best be judged by recording the level in the vegetation at which 'active' ticks were present. In the case of larvae and nymphs the vegetation clump was divided by eye into three sections, the top, middle and bottom thirds. Each third, starting at the top, was 'brushed' independently. In sampling the bottom third the fingers were firmly combed through the base of the clump. The recoveries at the three levels (Table 6) show that the proportion of active ticks taken from the top third was strikingly high.

The majority of adults, whose positions were noted individually, were also present within the top third (Table 6). Nevertheless, this observation, in which the position relative to the height of the clump as a whole was recorded, is not fully descriptive of their positions on individual stems, as some ticks were at the tips of shorter stems which were included within the middle third. These distances have therefore been measured. In 916 records of adults of both sexes the mean distance from the tips was 3 cm. 73 % of the ticks were within 0–3 cm. of the tip, 23 % were within 3–12 cm. and 4 % were within 12–34 cm.

Wind has a still more pronounced effect. In 370 observations, 207 adults (56 %) were on the leeward side of their stems, 104 (28 %) were intermediate and only fifty-nine (16 %) were on the windward side. This preference is very highly significant ($P = < 0{\cdot}01$). When the wind was stronger than B 1 on the Beaufort Scale 57 % of the ticks were found on the leeward side; 47 % were on the leeward with wind strength B 1–B 0.

Omitting observations when wind was B 0 and/or there was no sun, the distribution of observed ticks in relation to interplay of sun and wind was as follows (257 observations):

Position relative to wind	Position relative to sun: In	Intermediate	Out	Totals
In	23	10	7	40
Intermediate	23	10	38	71
Out	14	49	83	146
Totals	60	69	128	257

* Following R. A. Fisher's *Methods of Statistics*, chap. IV, Ex. 8.

The numbers intermediate to sun and to wind were about the same (69 and 71); and while the numbers out of the wind were about 3½ times those in the wind, the numbers out of the sun were only about twice those in the sun. This fortifies the suggestion above that though the tick tends to avoid both sun and wind, it is rather more concerned with avoiding the latter.

In the characteristic attitude of repose on the stem the capitulum may either be directed upwards ('right way up') or downwards ('inverted' position). Among males orientation is random (52 % inverted). Among females, however, there seems to be a distinct preference for the 'right way up' position (39 % inverted; $P = 0{\cdot}01$).

(iii) *Lateral movements*

The high percentage of recoveries (Table 1) suggests that there is little tendency for ticks to move away from the point at which they were laid down (see also Milne, 1950*a*). Although vertical movements along upright stems are characteristic, some lateral displacement occurs occasionally when the tick crosses from one stem to another in the tangled vegetation. The extent of this displacement was studied by marking certain prominent stems in each cage with coloured threads. The following are two typical examples.

(i) Female L/L first became active after 8 months of quiescence; the entire activity is included in the following sequence. The tick first climbed a 20 cm. grass stem *a* for 1 day, disappeared into the mat for 3 days, reappeared on a 30 cm. grass stem *b* for 2 days, disappeared for 12 days, reappeared on a 30 cm. rush stem *c* for 7 days, disappeared for 4 days, reappeared on *c* for 12 days, disappeared for 6 days, returned to *b* for 2 days, disappeared for 6 days, reappeared on *b* for 2 days. The distances separating the stem bases were *a*–*b* 5 cm., *b*–*c* 13 cm., *a*–*c* 20 cm. In this instance, access from one stem to another was probably gained along other stems that lay horizontally.

(ii) The activity of male –/L was confined entirely to two stems, a 30 cm. rush *a* and a shorter 20 cm. rush *b*. The phases of activity, in their correct sequence, were spent as follows: 3 days and 1 day on *a*, 1 day and 2 days on *b*, 6, 2, 7 and 2 days on *a*. The intervening phases of quiescence when the tick was absent lasted for 5, 2, 7, 5, 6 and 10 days respectively. The distance between the bases of *a* and *b* was 5 cm.

The movements or nymphs and larvae are also mainly vertical. At only one station (15) were ticks recovered outside the clump. Here many larvae were found swarming across the foot-wide circle of cropped turf that surrounded the clump. 894 larvae were recovered from the vegetation clump, while 239 were brushed from the cropped turf.

6. REACTIONS TO A 'HOST'

Previous experiments have shown that sheep ticks are attracted by warm objects if a favourable 'host' smell is also present. This is, of course, an oriented reaction, as the tick can sense the direction of the temperature gradient. In addition, alert ticks often respond vigorously to a sudden fall in light intensity (shading). This stimulus lacks the attribute of 'direction' and results in an unoriented response. Both these perceptions were believed to be of value in detecting the presence of the host and in orienting to it. While supporting these conclusions, confirmatory tests in the field have added some information both as to the mode of perception of the temperature stimulus and as to the general responsiveness of 'active' ticks at the vegetation tips.

For a few weeks the following tests were applied to all visible ticks that were resting with their forelegs folded: (*a*) the observer brought up his finger (the 'host') from the leeward side, then (*b*) from the windward side to within ½ cm. of the dorsum. He then lowered his notebook so as to throw an increasingly dense shadow (*c*); and finally brought up his hand, slightly cupped, from the leeward (*d*); then from the windward (*e*). A few similar tests were also made with pieces of fresh sheep wool attached to a length of wire.

The types of response evoked by the 'finger' and 'hand' tests (*a*, *b*, *d*, *e*), in order of increasing intensity, were as follows: feeble waving movements of the forelegs; quick, unoriented movements of the forelegs; vigorous oriented foreleg movements; excited 'rearing up' on the 3rd and 4th pair of legs, accompanied by oriented movements of the forelegs. All these were counted as positive responses. The 'shading' test also elicited a response varying in intensity. Some ticks failed to respond or moved the forelegs weakly, while others reared up and began to walk up and down their stems in an excited fashion. Tests with sheep wool never evoked any response.

Some typical results with individual ticks are given below. 0 denotes no response; otherwise the distance in cm. at which the stimulus first evoked a response is shown.

Tick no.	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)
1	0	0	0	0	4
2	0	1	12	0	17
3	0	1	7	0	22
4	0	0	4	0	0
5	0	15	0	0	18

It is noteworthy that the 'finger' and 'hand' tests were always more effective when applied from the windward, the cupped hand sometimes eliciting a response at 25 cm. As smell alone is ineffective, it

is clear that the tick is responding to eddies of air warmed by passage over the hand. As experimental evidence had also indicated (Lees, 1948), the thermal stimulus is evidently not perceived as radiant heat. Distance perception of warm air could also be demonstrated if the observer sat to the windward of a clump for some minutes. Active ticks often became alert and quested; it did not appear, however, that the proximity of the 'host' ever brought up fresh ticks from the vegetation base.

The routine tests showed also that the alertness of adult ticks in the resting posture was remarkably variable; many were so deeply 'asleep' that only a tactile stimulus would arouse them. In 826 tests 65% failed to respond at all to the finger from the windward and 88% to the finger from the leeward; and 73% gave no response to the shading test. If aroused at all, both stimuli were often effective. In 246 instances when both tests were applied to the same individuals, 58% responded to neither; 19% responded to both; 17% responded to the finger but not to shading; and 6% responded to shading but not to the finger.

7. DISCUSSION

(i) *The time relations of seasonal activity*

Since the review on seasonal activity in Britain (Milne, 1945*a*), additional observations have been made in Wales (Edwards & Arthur, 1947; Arthur, 1948), in Scotland (Campbell, 1950) and in northern England (Milne, 1945*b*; 1947*a*; also data in 1947*b* and 1949; and unpublished). The Welsh work has clouded the issues somewhat because of failure to recognize delayed stocking effects (cf. Milne, 1945*b*) and to allow for differential host-potential (cf. Milne, 1949). The subject will have to be reviewed again soon, but meanwhile the following generalization is considered sound (cf. Milne, 1948, 1949).

Throughout Britain tick activity mainly occurs either (1) in spring only, i.e. late March to June, with the peak usually in April or May, and only the merest trace of activity after summer; or (2) in both spring and autumn, the latter following a lull for a few weeks (usually in July) and covering the period August to early November, with the peak usually in September. Monannual activity appears to be confined to the eastern parts of the country (e.g. Northumberland and south-east Scotland), biannual activity to the western parts (e.g. Wales, Cumberland and west Scotland). In the case of the biannual type, the number of ticks active in autumn is invariably considerably lower than in spring provided host potential is about the same in both seasons. Hence, over Britain as a whole, spring is the main season of activity. (Incidentally, the prolongation of a considerable amount of 'spring' activity into July 1946 in our experimental populations (see Fig. 1) is due to absence of hosts—cf. delayed stocking effects in Milne, 1945*b*.)

Several suggestions have been offered in explanation of these regional differences in seasonal activity but when reviewed by Milne (1945*a*) none was found to fit the existing evidence satisfactorily. Despite the fact that the western climate is warmer and damper, it does not seem probable that the occurrence of autumn activity is directly controlled by it (although the possibility remains of a certain measure of *indirect* control through the timing of the developmental cycle—see below). Indeed, by suitably manipulating the emergence dates of an experimental population it is possible to create an autumn peak of activity in a 'monannual' locality (as at Hethpool—see Fig. 1), at a time, that is, when no activity is occurring in the surrounding 'wild' population.

It is now becoming apparent that seasonal activity is mainly a reflexion of the 'availability' of unfed ticks; or, in other words, of the presence or absence in the vegetation of an unfed population in the appropriate condition for activity. In a naturally infested locality the availability of such a population at any given season will depend primarily upon two factors. First, on the timing of the cycle of development; and secondly on the behaviour and possible survival of unfed ticks from season to season. The second point requires careful consideration as, under laboratory conditions, fasting ticks are extremely long-lived. Although the powers of survival of unfed ticks in nature have undoubtedly been exaggerated (p. 205), this factor must nevertheless enter into any interpretation of the time relations of the life cycle (see below).

A tentative reconstruction of the time relations of seasonal activity is outlined in Table 7. In this scheme results given in the present paper have been combined with Campbell's (1950) data on the development of the eggs and engorged stages in nature (shown by interrupted lines). The emergences of our experimental populations, with the exception of the October series, were not timed so as to provide a precise parallel with any particular natural population. Yet, with the reservations mentioned below, it is believed that their behaviour should be representative of a 'wild' population which has attained the same state of development in relation to the seasons.

Consider first the simple case of a population with a single spring activity peak, as at Hethpool (Table 7A). The spring-fed larvae, nymphs and females moult or lay eggs during the late summer and autumn (August–October) of year n, but low winter temperatures supervene before appreciable activity develops (Campbell, 1950). Activity begins in the spring of year $n+1$ when maximum day temperatures rise above the threshold for spontaneous activity (about 10° C.); and all surviving ticks take

Table 7. *A reconstruction of the life cycle in two localities where the populations display, respectively, spring* (A) *and both spring and autumn* (A + B) *activity*

The scheme shows the probable fate of these populations if no hosts were available after autumn of year *n*. The seasons are approximately as follows: 'spring', March–May; 'summer', June–August; 'autumn', September–October; 'winter', November–February.

	Year								
	n				*n* + 1				*n* + 2
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
A	*Engorged* Larvae	Moulting	*Unfed* Nymphs		–All active, die				
	Nymphs	Moulting	Adults		–All active, die				
	Females	Egg laying and hatching	Larvae		–All active, die				
B			*Engorged* Larvae		Moulting	*Unfed* Nymphs	–48 % active, die		–52 % active, die
			Nymphs		Moulting	Adults	–20 % active, die		–70 % active, die
							–10 % active		–Again active, die
			Females		Egg laying and hatching	Larvae	–97 % active, die		–3 % active, die

part in it during the ensuing weeks. The decline in activity in early summer is due to: (*a*) the diminishing numbers of available unfed ticks, as active individuals become fed; and to (*b*) the death of ticks that become worn out by activity after failing to achieve a host. As the work of Milne (1950*b*) has indicated that in nature the great majority of unfed females fail to find a host in spring even at the highest economic host-stocking densities, (*b*) may well be of major importance.

Even in the complete absence of hosts extremely few ticks, if any, survive into autumn and certainly none can survive into year $n+2$. It follows that the population remains virtually spring-active, the maximum and minimum span of the life cycle occupying 3 years. This interpretation corresponds with that given by Milne (1950*a*) and Campbell (1950).

The series laid down in October was intended to provide a parallel to this situation. Nevertheless, the behaviour of these ticks was evidently not entirely typical of the natural population for 76% of the larvae, 23% of the nymphs and 2% of the adults became active almost immediately, in the late autumn of 1945 (that is, in year n). The disturbance resulting from laying the ticks down in the vegetation was no doubt responsible, larvae being particularly sensitive to the breaking up of the clusters. With moulting and oviposition taking place *in situ* a much higher proportion of the activity would probably be deferred until the spring of year $n+1$.

In localities where biannual activity prevails, the time relations of the life cycle must be more complex (Table 7A and B). Ticks engorging in the spring of year n will of course follow the cycle indicated above (Table 7A). On the other hand, autumn-fed ticks will overwinter in the engorged state, the next unfed population appearing in early July of year $n+1$ (Campbell, 1950). These correspond approximately in time with our July experimental series. Now although these ticks were of a similar age, their behaviour was far from uniform. While some ticks in each stadium became active in autumn, yielding an autumn peak in year $n+1$, the remainder failed to appear until the spring of year $n+2$. The relative proportions (which are entered in Table 7) depend on the stadium: delay in the onset of activity was most marked in adults (70% exclusively spring-active), least marked in larvae (3% spring-active). If the behaviour of the July and May series is compared, it becomes apparent that the numbers which defer activity are influenced by the shift in the emergence date; but even in the May series, 34% of the adults and 1% of the nymphs and larvae were exclusively spring-active. It is noteworthy in this connexion that any disturbance associated with laying the ticks down could only hasten activity.

These results probably imply that in wild populations a proportion of the ticks of each stadium also fail to keep in step with the cycle of autumn activity. Indeed, constant reinforcement of the active spring population by individuals that have missed the preceding autumn season may account, in part, for the larger dimensions of the spring peak.

The life cycle of a tick which followed the autumn cycle exclusively would also occupy 3 years. At the same time, the cycle is unlikely ever to exceed 3–4 years in duration; for if activity in a larva or nymph is deferred until spring, in the way indicated above, spring activity will be continued by the succeeding nymph and adult.

Biannual activity is regarded by Campbell (1950) as resulting from the presence in the same environment of two entirely distinct populations, their development being such that spring or autumn activity is perpetuated in the respective populations. Judging, however, from the results with experimental populations of unfed ticks, it would be more accurate to regard these populations as only partially distinct, since during the spring season they will intermingle in varying proportions at each level of development.

On these grounds it would seem that the monannual spring cycle represents the more stable condition. In localities showing the typical biannual cycle, autumn activity might be expected to dwindle and even to disappear entirely unless autumn-active populations were in some way reinforced. Our present results do not provide any hint as to the nature or significance of such a counterbalancing factor. It may be, however, that the warmer climatic conditions of the west lead to an acceleration of development and to the appearance of appreciable autumn activity in a spring-fed population, especially in unusually hot summers.

(ii) *Individual activity and survival*

Unfed females have been shown to spend an average of only 9 days at the vegetation tips during an active life of 30 days. There is reason to believe that in many instances the duration of survival is also unexpectedly short.

Experiments in which ticks could be recovered after spontaneous activity had ceased (Lees, 1950), showed that death rapidly supervened after the last day of activity. If such is also the case under natural conditions, active ticks in nature must be short-lived in comparison with individuals maintained in the laboratory under conditions which effectively prevent activity. The following examples illustrate this principle. Females from stations 1–4 that were active only in 1945 survived for an average (estimated) period of 13 weeks; those becoming active first in 1946 survived for 51 weeks. On the other hand, a similar group confined in a small tube (in saturated air at 11° C.) survived for an average of 85 weeks. A further instance of the reduced longevity

—in spring-active female ticks—has been recorded by Milne (1950*a*).

The explanation is that at emergence the tick possesses a fixed reserve of fat. During periods of inactivity at the vegetation base these reserves are drawn on very sparingly but are quickly exhausted once activity commences (Lees, 1950). Length of survival therefore depends mainly on whether activity begins soon or late after emergence. Whichever occurs, the potential longevity can never be realized.

One practical outcome is that it is comparatively rare in nature for one individual to remain in the unfed stage during more than one calendar year (cf. Milne, 1950*a*). Similar observations have been made on *Dermacentor andersoni* (Philip, 1937) and *D. variabilis* (Smith, Cole & Gouck, 1946). In the second species, as in *Ixodes ricinus*, active ticks captured late in the season show a 'reduced vitality'. In *Ixodes* this is manifested by the lowered resistance to desiccation as well as by the diminished expectation of life (Lees, 1950).

Although in the sheep tick actual locomotion is no doubt partly responsible for this 'exhaustion', the impression gained is that the total movements are extremely small, most of the active life of the tick being spent at rest near the vegetation tips. However, the responsiveness to 'host stimuli' of ticks in this situation is clearly far higher than that of quiescent individuals at the vegetation base. This has been brought out by sampling the vegetation at different levels, for very few of the ticks known to be present within the bottom third of the vegetation ever responded to the hand. It may be that the maintenance of an alert wakeful attitude at the tips itself involves heightened metabolic activity and an accompanying drain on the reserves.

The effects of activity on survival are also relevant in interpreting the results of 'delayed stocking'. Milne (1945*b*) has shown that if sheep were withheld from an infested hill grazing until 0–3 weeks after the normal spring peak, no appreciable prolongation of activity into the summer months occurred. This experiment, as well as subsequent work (Milne, 1946) in which infested plots were systematically dragged with the blanket twice daily throughout the tick season ('exhaustion dragging'), demonstrated that some adults and nymphs become active each week for the first time, and led to the inference that the average duration of activity in a natural population of ticks was short (for female ticks 'probably not more than a month, perhaps less' (Milne, 1945*b*)). These deductions are amply confirmed by the present results which enable a further point to be added. The short active life of 30 days arises not only because the total amount of activity (days at the tips) is small but because activity when once commenced usually proceeds with only short intervals of quiescence until the tick dies through exhaustion.

These facts may now be placed in relation to 'delayed stocking'. In the experiments cited above it is clear that by the time sheep were admitted to the pasture, 6–8 weeks after the normal onset of spring activity, a substantial proportion of the total population would have gone through their entire activity and died. It was estimated that less than half the female ticks available were actually fed by the end of the season. These would consist of females whose active life was not yet over when the stock was readmitted, and females that became active for the first time during the latter part of the activity season. From this it is evident that in nature the rapid utilization of the reserves during activity must severely limit the opportunities for host-finding.

(iii) *The sensory perceptions in the natural environment*

In finding and attaching itself to a host, a sheep tick would be expected to encounter various guiding stimuli of a physical or chemical nature. In a previous study of the sensory physiology the reactions to such stimuli, as observed in the laboratory, were assigned a provisional significance (Lees, 1948). In the light of additional experience of the behaviour in the natural environment several points require further discussion.

These stimuli are probably presented in a definite order. (i) The first series were regarded as responsible for guiding the tick to a position of vantage near the vegetable tips where the host is likely to be encountered. (ii) The second series provide a warning of the approach of the host and when the host is close at hand can serve for orientation. (iii) The third series of stimuli lead to the attachment response when the tick has transferred itself to the host.

With regard to (ii) and (iii) little need be added to the previous account. Routine tests with a human 'host' have confirmed the effectiveness of a sudden reduction in light intensity (shading) in causing instant alertness. Other stimuli which possess the quality of direction induce alertness and then elicit vigorous orientation. The relevant stimuli here are air temperature and perhaps smell.

With regard to (i), on the other hand, a consideration of the significance of the gravity and humidity reactions raises some interesting but difficult questions. To what extent do these operate in nature? What is the contribution of each in guiding the tick to the vegetation tips? Finally, what is the value of the humidity behaviour as a safety device?

Such observations as have been made on ticks climbing the vegetation, demonstrate that the gravity reaction, which involves upward-turning near the tips, is very commonly displayed in the field and is of primary importance in causing the tick to settle near the tips.

Previous experiments have shown that there are two entirely different reactions to humidity, related to the state of the water balance. Ticks with a normal water content at first avoid damp air when exposed to a humidity gradient. In the natural environment where a high humidity prevails at the vegetation base and a falling gradient extends upwards towards the tips, this behaviour might also tend to induce upward-turning. The field observations, however, provided little indication that this reaction ever comes into play unless the gravity reaction fails, and this appears to be infrequent.

A further suggestion concerned the survival value of the humidity behaviour. The experimental finding was that after desiccation the reaction (a taxis) described above is replaced by a second mechanism (a kinesis). The tick is then constantly active in dry air but soon comes to rest in moist air which, of course, it no longer avoids. It was argued that after an unsuccessful period of waiting at the tips, progressive desiccation would force the tick to leave the tips, walk down to the moist microclimate of the mat and come to rest. The quiescent phase would then provide an opportunity for restoring the depleted water balance, in preparation for a further cycle of activity.

Fig. 3 shows that it is, in fact, unusual for ticks to remain continuously at the vegetation tips throughout the active period.* Where a series of long phases at the tips alternate with short disappearances of a day or so, the pattern of activity may well be regulated by the humidity behaviour. One day allows sufficient time for the tick to regain any lost water (Lees, 1946). In laboratory experiments a water loss equal to about 12% of the original weight was needed before unfed females at rest were stimulated to activity. However, active ticks in the field are usually far more alert than laboratory specimens, and it is conceivable that a smaller water loss would produce the critical desiccation required.

Unfortunately, it would be exceedingly difficult to prove that in any given instance the kinesis had operated. 'Spontaneous' movements, which doubtless account for the irregularity of many individual activity patterns, are the main source of confusion. Certainly the kind of humidity behaviour outlined above cannot be involved in the very short phases of activity, often no longer than 1 day, that occur in the records of many very active individuals. This period is too short for the critical desiccation to have set in, even with unusually low air humidities.

Assuming that the humidity behaviour serves, on occasion, as a safety device for preventing excessive desiccation during protracted spells of activity, one may further inquire whether the regulation of the up and down movements enables the tick to remain at the tips for more days (in aggregate) than would otherwise be possible. In experiments by Milne (1950*a*), in which captured wild female ticks were suspended in silk envelopes at the vegetation tips, the average period of survival was 4–5 weeks (although it is pointed out that this was a time of abnormally high humidity). Now the average number of days each tick spent at the tips in our experiments was 9 days and values above 30 days were exceptional. On this basis there would appear to be little gain, except perhaps that the active phases are spread out over a longer total active period. It is a curious fact, therefore, that the survival value of this behaviour, in this situation at least, seems to be largely neutralized by the physiological effect of activity in curtailing the total life span.

It must be remembered, however, that the unfed tick often spends many months at the vegetation base before actively seeking a host. Although the 'mat' microclimate as a whole remains close to saturation throughout the year (Milne, 1950*a*), localized pockets might well occur where the humidity fell below saturation during the summer months. Survival would depend on the avoidance of such situations.

* Other species of ticks living amongst vegetation, e.g. *Dermacentor andersoni* (Philip, 1937) and *D. variabilis* (Smith, Cole & Gouck, 1946) evidently behave similarly.

8. SUMMARY

1. Activity in *Ixodes ricinus* was studied by laying down newly emerged ticks in natural clumps of vegetation from which all hosts (with the possible exception of 'mice') were excluded. After a period of quiescence the ticks climbed to the 'active' position at the vegetation tips. Activity was assessed either by simple observation (in adults which were marked individually) or by 'brushing' the vegetation with the hands (nymphs and larvae). 52% of the adults and 44% of the nymphs were recovered.

2. The seasonal activity behaviour was followed in three series of ticks set out during May, July and October 1945. The results were used in interpreting the time relations of seasonal activity in 'wild' populations. The timing of the activity cycle appears to be largely determined by the availability of unfed ticks rather than by the prevailing meteorological conditions.

3. The diurnal pattern of behaviour in active ticks was closely examined during a 24 hr. period. Many remained at the tips without moving. The others began or ended a phase of activity by night or by day; but among these, the proportion ending a phase at night was significantly greater. This is the main reason for the previously reported diurnal fluctuation of activity in a tick population.

4. Two hundred and seven adult ticks spent, on an average, a total of 9 days at the vegetation tips (individual limits 1 and 54 days). This activity was

spread over an 'active period' (interval between first and last appearances) averaging 30 days. Very active ticks rarely remained continuously at the tips. The mean number of visits to the tips was 4 (limits 1 and 19), each lasting for an average of 2·5 days. Where long spells of activity alternate with short periods of quiescence, the behaviour is probably regulated by the humidity reaction, the quiescent phase providing an opportunity for restoring the depleted water balance.

5. Newly moulted adult ticks possess reserves of fat adequate for many months of quiescence. During activity these reserves are exhausted in a few weeks or even days. Duration of survival mainly depended on whether the onset of activity was immediate or delayed. Some adults remained quiescent for 1 year before becoming active for the first time.

6. Several types of orientations were observed in the field. In attaining the position at the tips favourable for encountering a host, the gravity response (upward-turning near the tip) is of major importance. The tick avoids wind and direct sunlight by sheltering behind its supporting stem. In sensing the approach of a 'host' (the observer's finger) the perception of eddies of warm air is particularly significant. The response (questing, then orientation) is elicited much less readily if the stimulus is applied from the leeward.

Our warmest thanks are due to Mr T. P. McNicol, Assistant Experimental Officer, who painstakingly carried out rather more than half the routine observations each week during April–June in 1945 and 1946.

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THE SHEEP TICK, *IXODES RICINUS* L.

ERADICATION BY DIPPING SHEEP

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INTRODUCTION

Nuttall (1911, 1916) records the British tick, *Ixodes ricinus* L., as occurring in Europe (nearly all countries), North Africa (Algeria, Tunisia), North America (Canada and U.S.A.), North Arabia, Transcaucasia, Kashmir and Japan. In Great Britain, it is confined mainly to hill pastures and is a source of considerable loss to flockmasters. Until 1930, loss of blood and irritation due to tick bites was thought to be the main damage resulting from tick infestation, although it was realized that the tick is the vector of *Babesia bovis*, the cause of British bovine piroplasmosis.

Gordon, Brownlee, Wilson & MacLeod (1932) finally showed the tick to be the vector of louping ill, an encephalomyelitis of sheep. Stockman (1918) failed to recognize the transmission of louping ill by ticks because the ticks which he used appear to have been carrying the infective agent of tick-borne fever, a disease which was shown by Gordon *et al.* (1932) to be clinically and immunologically distinct from louping ill. Although mortality due to tick-borne fever appears to be negligible, it is thought to be responsible for serious setbacks in lambs on tick-infested farms and may cause abortion in non-acclimatized ewes brought on to these farms. Macleod (1939*a*) states that louping ill is not generally a problem in acclimatized stock, but that up to 100 % of non-acclimatized stock may die and a 75 % mortality is not unusual. A survey of farms in Cumberland has not confirmed this, but losses of up to about 50 % have been reported in this area. Lyle Stewart (1939) records 230 deaths from louping ill in a flock of 283 unacclimatized sheep within 6 months of their being brought to a tick-infested pasture. Occasionally, louping ill causes severe losses in cattle.

Tick paralysis, a disease which is frequently fatal, results from attachment of some species of ticks to many animals, including man, and Macleod (1939*a*) cites a case in Crete which was believed to follow the bite of *Ixodes ricinus*.

A pyaemic condition of hill lambs is believed to be associated with tick bite. During 1947, the only year for which records were kept, 40 (11·4 %) of the 350 lambs born on a farm in Cumberland showed the 'cripple' syndrome of this disease and, of these, 13 (32·5 %) died. McDiarmid (1946) has shown that this disease can also occur as a rapidly fatal *staphylococcal* septicaemia, so that the true incidence of the disease may be higher than the above figures indicate.

Apart from specific diseases carried by ticks, it has often been stated (Macleod, 1938, 1939) that ticks cause damage to sheep by irritation, and primary anaemia due to removal of blood. It is very difficult to assess the degree of irritation caused by ticks, but observation of sheep which are heavily infested has never yielded any signs that the tick causes more than slight irritation. Small inflammatory reactions

often occur at the site of tick bite in lambs. Exact figures for removal of blood are also difficult to obtain. Macleod (1939*a*) concluded that the tick injects an anticoagulin into the wound, basing this assumption on the fact that 'during some work on tick rearing large quantities of dried blood were repeatedly found as a fine powder in the infestation bag, which was attached to the scrotum of the sheep'. The supposition that this represents blood draining from the wound after the tick detaches is entirely unjustified, as the engorging tick passes blood through the alimentary canal almost unchanged prior to the actual process of engorgement (Lees, 1943). Further information on the amount of blood removed by ticks is contained in the Appendix.

Macleod (1939*a*) states that burst ticks cause bacterial activity and lead to neck and shoulder strikes, but this statement should be accepted with reserve until supporting evidence is presented, as myiasis of the neck and shoulder is common in tick-free areas of Great Britain.

Of 458 sheep which died on a hill farm in Cumberland during the 5-year-period, 1943–7, twenty-five are known to have died from the effects of the pyaemia associated with tick bite, and louping-ill caused five deaths. Cause of death was not determined in 141 of these sheep, but it is probable that some died as a result of the staphylococcal septicaemia which may follow tick infestation.

Jamieson & Soltys (1947) suggest that an infectious epididymo-orchitis of rams associated with *Pasteurella pseudotuberculosis* may be transmitted by ticks.

Thus, although it would be difficult to make an accurate estimate of damage caused by tick infestation of sheep, it appears that the tick may be classed as one of the major parasites of sheep in Great Britain. Eradication presents some difficulty because, during the 3 years occupied by the life cycle, only 15–20 days are spent on host animals. The remainder of the time is spent in moist layers at or near the surface of the soil, or on top of the vegetation waiting for a passing host. Mail (1942) states that treatment of pastures with acaricides is too expensive to be practicable and, in Great Britain, the character of the countryside frequented by ticks precludes this method of attack. Burning the pasture kills only those ticks which happen to be on the herbage proper, and probably has little effect upon the ticks which are in the soil or in the moist layer of vegetation covering the soil; it has been shown to be of little value as a means of tick eradication. Unless conditions permit radical alteration of its habitat (e.g. by ploughing up old grassland) control measures must be directed to killing the tick while on the host animal.

Among domestic animals, cattle and sheep are the principal hosts of *Ixodes ricinus*, but Macleod (1932) compiled the following list of alternative hosts: horse, deer, hedgehog, dog, cat, fox, stoat, weasel, ferret, hare, rabbit, rat, squirrel, hoodie crow, jackdaw, golden plover, grouse, kestrel and merlin hawk. Only immature stages were found on birds. Milne (1947*a*), however, found that, on pastures from which cattle, deer and hare are absent, about 98 % of adult ticks are fed by sheep. The present work was concerned only with measures to prevent ticks from attaching on sheep.

From time to time, farmers have reported that incorporation of certain substances, notably sulphur, aloes and garlic, in the diet of host animals gives protection from tick infestation, but trials to investigate these claims have given negative results (Mail, 1942). Milne (1947*b*) states that sheep in poor condition harbour more ticks than do those in good condition, but it will be seen from the text that this finding has not been confirmed. The most popular method of keeping sheep free from ticks has been by treating the skin and fleece with chemicals which are known to be lethal to ticks. Dipping is at present the usual method of applying the acaricides, and it is with evaluation of existing and improved dips that the present paper is mainly concerned. Milne (1945) concluded that: 'dipping has a general beneficial effect partly due to rendering the sheep free from other parasites, and partly due to the tonic effect of a cold bath'. No direct experimental evidence was offered in support of this statement and, during 8 years' extensive experience of field trials of dips by the present writer, no evidence has been gathered to support this contention as a whole. Whilst dipping against a parasite such as the sheep louse would almost certainly benefit the health of the sheep, it is suggested that 'the tonic effect of a cold bath' may be disregarded. Any apparent improvement in condition of sheep which are not heavily infested with ectoparasites may safely be ascribed to the fact that a clean sheep looks more attractive than a dirty one. Until early in the present century, hand smearing of the fleece, which is recorded in Great Britain as far back as A.D. 1280 (Duncan, 1946) was extensively practised as a method of tick control, but has become almost obsolete. Treatment of the fleece with dusts, notably derris, now has a limited application, and the possibility of a spraying technique to replace dipping is being investigated.

MATERIALS AND METHODS

Prior to 1945, commercial anti-tick dips in Great Britain had for many years consisted of mixtures of arsenic, cresols and grease, with slight variations. The commercial dip to which reference is made in this work was of this type and, unless otherwise stated in the text, the same commercial dip was used in every case.

(a) Early methods of testing anti-tick dips

In early field trials, two or more groups of sheep were dipped and turned out to graze on tick-infested pasture, with or without undipped controls. At about weekly intervals, thereafter, ticks were counted on each group, and arithmetic means of the group counts were compared by means of the 't' test (Fisher, 1932). During tick counting, only those females attached on the face, ears, axillae and groins were recorded. A few ticks are present in the fleece proper, but observations showed that these find great difficulty in escaping after engorging as they become entangled in the wool fibres and so die; for practical purposes, they may be disregarded and, in any case, accurate counting of these is almost impossible. This method was used by Macleod (1939*a*) who states: 'except for a few attached to the bare parts of the abdomen, the ticks on these areas represent practically the entire female population on a full-fleeced sheep'. Of 24,388 attached female ticks counted on 1248 undipped sheep in Cumberland, 47 % were on the ears, 18 % on the face, 22 % on the axillae, and 13 % on the groins. It has been found impossible to count accurately nymphs and larvae. At an early stage in field trials of anti-tick dips, it was felt that the classical method of tick counting has many limitations. Chief of these is the fact that, if a dip did not kill ticks until after they had been attached for several days, many of the ticks which were counted during routine counts might be doomed to die before engorging and thus be of no importance from the standpoint of tick eradication. Milne (1945) realized this, but failed to suggest any means of improving the method. Blaxter (1946) showed statistical comparison of counts to be of little use unless more detailed field observations are made.

(b) An improved method of testing anti-tick dips

After many fruitless attempts, which included an effort to mark all freshly attached females with cellulose paint, changing the colour daily, a method was evolved whereby a record is made of each female tick which attaches on the sheep and its subsequent fate is noted by daily observation. This method has now been adopted by the Scottish Agricultural Advisory Council as the standard method of comparison of anti-tick dips. Up to 4 days, the length of time for which a female tick has been attached to a sheep can be judged by an experienced operator with a fair degree of accuracy. According to Macleod (1932), females normally engorge in 7–10 days, but observations prior to, and during, intensive tick watching indicated that a proportion may engorge in 5 days. It is upon these two facts that the improved method of dip testing is based. An exact description of the technique of recording would be cumbersome, but the method has shown itself to be satisfactory, and it is now possible to record:

(*a*) Number of females which attach to the sheep.
(*b*) Number of females which die on the sheep.
(*c*) Number of females which disappear before engorging (some of these probably die before dropping off).
(*d*) Number of females which engorge.

Since ticks which do not completely engorge lay no eggs, or very few eggs (Campbell, 1945), and because a period of at least 5 days appears to be necessary for complete engorgement, any tick which remained on the sheep for less than 5 days was considered to have failed to engorge, thus causing a break in the life cycle; during interpretation of results, these ticks were considered to have died. By use of this detailed observation of ticks, it is considered that groups of six sheep are adequate for comparison of acaricides.

(c) Variation in individual susceptibility to infestation

Individual sheep show marked variation in their susceptibility to tick infestation; the following ranges encountered during tick counts on fifty undipped sheep running on the same pasture between 13 March and 2 July give some indication of the variability: 0–1, 0–4, 0–15, 0–20, 0–23, 0–35, 0–33, 0–17, 0–10, 0–21, 0–59, 0–16, 0–5, 0–5. It will be seen from the text that the reason for this phenomenon is not yet understood. When comparing anti-tick dips by the usual method, it is essential to dip large groups of sheep in order to eliminate errors due to this factor.

(d) Speed of infestation of undipped sheep

Also, if sheep were dipped in an acaricide which had no tick-repellent properties, it follows that an interval would occur between their return to tick-infested ground and the time when they attained the level of tick infestation of sheep already on the pasture. To discover how long this interval is likely to be, forty undipped sheep from a tick-infested pasture were randomized into groups of twenty. To simulate treatment which was acaricidal but not repellent, all female ticks on areas to be counted were removed by hand from one group, and both groups were then returned to the pasture. Tick counts on these sheep are shown in Table 1. The experiment

Table 1. *Speed of re-infestation following removal of ticks from sheep by hand*

	Days after returning to pasture		
	1	3	6
Deticked	2·7*	5·6*	9·1
Control	11·7	10·4	11·3

* Significantly lower than control group.

was repeated, results being shown in Table 2. An apparent repellent effect may persist for up to 6 days, and comparison of counts during the 6 days after dipping will be of little value.

Table 2. *Speed of re-infestation following removal of ticks from sheep by hand*

	Days after returning to pasture			
	1	2	5	6
Deticked	1·1*	5·7*	9·3*	10·1
Control	13·6	15·1	16·3	15·2

* Significantly lower than control group.

RESULTS

(*a*) *Grazing habits of heavily infested sheep*

Heavily infested individuals might be those which frequent the more 'ticky' portions of the pasture. That this explanation is, at most, only correct in certain cases is shown by the following result. Four Herdwick sheep of similar fleece type, and all in good condition, were placed on a 30-acre enclosure and tick counted on thirty-nine occasions during the following 40 days. They appeared to graze together throughout the day and, while being driven to the pens for tick counting, covered the ground very thoroughly. Numbers of attached female ticks counted were: 319 on sheep no. 1, 316 on no. 2, 662 on no. 3, and 634 on no. 4. Sheep nos. 3 and 4 were consistently more heavily infested than nos. 1 and 2.

(*b*) *Effect of condition of sheep on susceptibility to infestation*

Macleod (1932) found that 'variation in the liability of individual sheep to infestation appears to be correlated with the condition of the sheep'. On evidence obtained from one count on three sheep, he confirmed a shepherd's observation that pregnant or nursing ewes are often more heavily infested than the better conditioned barren ewes. Milne (1947*b*) presents evidence to show that sheep in good condition carry fewer ticks than do those in poor condition. In the present work, every time a large number of sheep was brought into the pens, ticks were counted on the markedly unthrifty sheep and on the same number of fat sheep. Only 116 really thin sheep have been available, and they yielded a total of 1940 attached females; on 116 fat sheep, 1964 females were counted. To obtain further data on this point, seven undipped sheep in good condition, together with seven sheep in poor condition, were placed in a 30-acre intake. Condition of the sheep was assessed independently by the shepherd and the operator, the two opinions coinciding in every case. Attached females were counted on these sheep on twenty-four occasions during the following 54 days. A total of 2460 ticks were counted on the fat sheep, and 2738 on the thin sheep. Differences between the means of the group counts were in no case significant. The writer suggests that any apparent predisposition to infestation by ticks shown by thin sheep is due to the fact that, concurrently with loss of condition, loss of wool frequently occurs, and this renders attached ticks more easily visible.

(*c*) *Effect of grease content of wool on susceptibility to infestation*

Macleod (1932) suggests that susceptibility to infestation may vary inversely with the grease content of the fleece. To investigate this point, two undipped Herdwick ewes of similar fleece type and condition were placed in a 30-acre enclosure and, on sixteen occasions during the following 39 days, a total of 246 attached females was counted on ewe no. 1, while 671 were counted on no. 2. Wool samples taken during the course of the experiment and extracted with chloroform in a Soxhlet apparatus showed a grease content of 8·0 % in ewe no. 1 and 4·6 % in ewe no. 2, thus indicating the possibility of Macleod's suggestion being correct. On another occasion, however, seven undipped sheep of similar condition, but with grease contents of the fleece differing widely, were placed in a 30-acre enclosure and tick counted twenty-two times during a 46-day period. It will be seen from results shown in Table 3 that no obvious correlation existed between grease content of the fleece and tick infestation.

Table 3. *Tick counts on sheep and grease contents of their fleeces*

Sheep	Fat content of fleece* (%)	Mean tick count
1	4·0 (3·7)	25·6
2	6·4 (2·5)	24·0
3	7·1 (2·7)	13·2
4	7·4 (2·8)	34·1
5	9·3 (4·8)	22·6
6	12·0 (2·4)	28·4
7	13·0 (1·3)	20·4

* Percentage suint content shown in parentheses.

Although the groins offer the largest hairless area on the sheep's body, relatively few ticks attach there. A possible explanation of this lies in the thick layer of grease which generally overlies the skin in this area. After being freed from ticks by hand picking, the groins of five undipped sheep were well washed with a solution of Cetavlon (cetyltrimethylammonium bromide) in water, leaving them apparently free from grease. Only the right groins of a further five sheep were similarly washed, after both groins had been deticked. These ten sheep were then placed in a pasture along with ten untreated sheep from whose groins ticks were not removed. Table 4 shows the results of eleven tick counts during the following

22 days. Removal of grease did not appear to encourage tick attachment, and this was further borne out by the following observation on a sheep in the undipped group during a field trial of anti-tick dips during the springtime. Owing, presumably, to ill-health, this sheep cast its coat very cleanly, leaving the body surface naked except for a light covering of 'down'. During the next 20 days, infestation of this sheep was among the lowest of the undipped group of twenty sheep.

From the foregoing observations, the only firm conclusion which can be drawn is that individual sheep differ widely in their susceptibility to tick infestation, but no evidence has yet been advanced to explain this phenomemon. This is in substantial agreement with Milne's (1947*b*) findings; he suggests, further, that individual activity on the part of the sheep probably influences the number of ticks picked up.

Table 4. *Tick infestation of groins after removal of grease. Mean tick counts*

			Grease removed from right groin		
	Control	Both groins degreased	Left groin	Right groin	Total
Before treatment	3·3	4·0	1·8	2·5	4·3
1 day after treatment	4·6	1·8	0·3	0·7	1·0
2 days after treatment	3·4	2·0	1·3	0·0	1·3
3 days after treatment	6·3	3·7	1·0	1·5	2·5
4 days after treatment	6·0	5·8	2·7	2·3	5·0
9 days after treatment	5·5	11·8	4·0	3·4	7·4
10 days after treatment	4·6	9·0	1·3	2·7	4·0
11 days after treatment	4·8	6·8	4·3	2·5	6·8
14 days after treatment	4·1	6·4	2·0	1·6	3·6
16 days after treatment	3·3	4·4	2·0	1·0	3·0
19 days after treatment	3·0	5·5	1·0	1·0	2·0
22 days after treatment	4·1	2·3	1·0	3·5	4·5

Table 5. *Repellency of arsenical dips. Mean tick counts*

	Days after sheep were deticked by hand, 10 days after dipping			
	2	3	5	6
Commercial dip	2·0	2·2*	2·9*	5·0*
Undipped	4·0	8·8	8·3	10·0

* Significantly lower than control group.

Table 6. *Repellency of arsenical dips. Mean tick count*

	Days after dipping							
	5	7	10	14	17	23	31	37
Commercial dip	0·5*	0·5*	0·5*	1·0*	4·0*	7·5*	4·5*	5·0
Undipped	19·0	12·0	7·5	5·5	8·0	13·5	9·0	4·5

* Significantly lower than control group

Table 7. *Repellency of arsenical dips. Mean tick count*

	Days after dipping											
	7	10	14	17	22	25	29	32	36	40	43	46
Commercial dip	0·0*	0·5*	1·0*	2·5	4·0	7·0*	4·5*	6·0	3·5	1·0	1·0	1·5*
Undipped	15·0	9·0	3·5	2·5	5·0	14·5	10·0	8·0	3·5	2·0	4·0	2·5

* Significantly lower than control group.

(*d*) *Arsenical dips*

The commercial dip proved to be an excellent acaricide and prevented re-infestation for from 3 to 5 days, but it was considered that a partial protection remained even after ticks began to attach on the dipped sheep. To investigate this point, twenty

sheep which had been dipped in the commercial dip 10 days previously were deticked by hand, and all ticks were similarly removed from twenty sheep which had not been dipped for several months. Unfortunately, the experiment had to be abandoned to fit in with farm management, but tick counts during the following 6 days (Table 5) showed that some protection from infestation remains for at least 16 days after dipping. This finding was confirmed on many subsequent occasions. Tables 6–8 show results taken at random from routine field trials. The reason for the variation in time for which this 'residual' protection operates is not clear, but it is suggested that, when ticks are very active in seeking hosts, they will attach on dipped as readily as on undipped sheep. Thus, in Tables 6 and 7, infestation of control sheep was falling during the 3 weeks immediately following dipping, while, in Table 8, infestation of control sheep remained high.

Table 8. *Repellency of arsenical dip. Mean tick count*

	Days after dipping		
	7	13	18
Commercial dip	3·0*	11·0	17·0
Undipped	9·0	15·0	14·0

* Significantly lower than control group.

(*e*) *Comparison of sodium arsenite with commercial arsenical dips*

The reason for combining arsenic with cresols and wool grease in commercial dips is not clear. Milne (1945) states: 'One of the chief functions of the cresols is to prevent decomposition of the grease' but presents no evidence to support this statement, and it is unlikely that any one specific component is required to prevent decomposition in an arsenical dip. The function of grease is not clear, but it has been claimed by Macleod (1941) and Milne (1945) that it prolongs the effect of sodium arsenite; supporting evidence was not produced by either worker. In this work, the commercial dip was tested against a solution of sodium arsenite in water, with no additional wetting agent. Thirty sheep were used in each group and tick counts obtained are shown in Table 9. The experiment was repeated with the results shown in Table 10. Both dips killed satisfactorily and both gave the same measure of protection against re-infestation.

(*f*) *Comparison of arsenical with arsenic-derris dips*

Macleod (1941) incorporated derris in an arsenical dip and claimed 'protection of a very high order because ticks are killed by derris before they can attach'. Since no evidence is offered in support of this statement, it is presumably based on the assumption that arsenic is solely a stomach poison. Burtt (1945) showed that sodium arsenite is a contact poison to ticks. In the present work, a group of eighty sheep were dipped in Macleod's arsenic-derris dip, and eighty more sheep were dipped in the commercial dip. Tick counts are shown in Table 11, and results obtained in a repeat trial appear in Table 12. It appears that derris does not appreciably affect the protective effect of an arsenical dip.

Table 9. *Comparison of commercial arsenical dips and sodium arsenite as anti-tick dips. Mean tick count*

	Days after dipping		
	9	15	21
Commercial dip (0·22 % As_2O_3)	2·6	16·5	43·3
Sodium arsenite (0·2 % As_2O_3)	1·9	10·2	32·6

Table 10. *Comparison of commercial arsenical dip and sodium arsenite as anti-tick dips. Mean tick count*

	Days after dipping						
	5	8	10	12	14	17	20
Commercial dip (0·22 % As_2O_3)	0·7	0·7	1·1	2·5	2·6	2·2	10·1
Sodium arsenite (0·22 % As_2O_3)	0·3	0·7	1·7	2·6	3·0	3·5	6·0

Table 11. *Comparison of arsenic and arsenic plus derris as anti-tick dips. Mean tick counts*

	Days after dipping	
	14	21
Commercial dip	24·6	37·1
Macleod's dip	32·0	28·7

Table 12. *Comparison of arsenic and arsenic plus derris as anti-tick dips. Mean tick counts*

	Days after dipping		
	7	13	18
Commercial dip	0·2	10·0	6·8
Macleod's dip	0·1	10·1	7·0

(*g*) *Harmful effects of frequent dipping in arsenic*

Ministry of Agriculture 'double dipping' regulations are presumably based on the belief that it is dangerous to repeat dipping in arsenical dips at short intervals. There appears to be no published evidence to support this assumption, but Duerden, Maré & Bosman (1933) dipped thirty sheep in sodium arsenite at a bath concentration of 0·2 % As_2O_3 thirty-nine times in 1 year without producing any ill-effects. Hill (1946) dipped four sheep in a bath of

sodium arsenite at a concentration of 1·06 % As_2O_3 with no ill-effects. In the present work, groups of up to thirty sheep have frequently been dipped at weekly intervals in full-strength arsenical dips without any harmful effect arising. On the other hand, of seventeen lambs which were dipped (by accident) in a bath of arsenical dip at a concentration of approximately 0·4 % As_2O_3, eleven died within 2 days and all developed skin lesions, with symptoms of acute pain. Also, Harbour (1949) states that harmful effects have frequently followed short-interval dipping in full strength arsenical dips (0·2 % As_2O_3) but that these cases have escaped publication.

(*h*) *Coal-tar fractions*

During the search for an efficient anti-tick dip, a series of coal-tar fractions, in some cases combined with other active ingredients, were tested in the field on groups of twenty sheep. None of these proved to be better than the commercial dip and many were neither acaricidal nor repellent. For the sake of brevity, the list of substances tested, together with brief remarks upon results achieved, have been collected into a table and included as Appendix 2.

(*i*) *Finely divided inert powders as acaricides*

Chiu Shin Foon (1939) showed inert powders to be lethal to the bean weevil, *Acanthoscelides obtectus* Say, but a trial of finely divided alumina, strontium sulphate, Neosyl, barium carbonate and silica as acaricides gave negative results.

(*j*) *D.D.T. as an anti-tick dip*

In 1943, D.D.T. became available and *in vitro* toxicity tests showed it to be an efficient acaricide (Burtt, 1945). Results of preliminary field trials of this substance were published by Heath & Mitchell (1946). By dipping in D.D.T. at a bath concentration of 0·5 % five times during the spring tick season, Heath (1946) prevented any female ticks from engorging on six sheep. This result could probably have been achieved by a smaller number of dippings, and the following experiment was planned to investigate this point. A few days after ticks first appeared on sheep in the spring of 1947, eighteen hoggs were randomized into three groups of six, which were then treated as follows:

Group 1. Undipped.

Group 2. Dipped in the commercial dip.

Group 3. Dipped in M 37 (Heath & Mitchell, 1946) at a bath concentration of 0·5 % D.D.T.

Thereafter, it was planned to repeat the dippings of groups 2 and 3 as soon as ticks began to engorge on any sheep in the group, i.e. when a female tick remained attached for 5 days and showed no sign of imminent death. In group 2, repeat dippings were found to be necessary 16, 28, 45, 58 and 75 days after the initial dipping, while group 3 was redipped 28 and 58 days after the initial dip. Arithmetic means of group tick counts at 5-day intervals are shown in Table 13, and Table 14 summarizes results of intensive tick watching. Thirty-four of the ticks recorded as 'engorged' in group 2, and two of those in group 3, detached overnight following dipping. It is probable that these ticks died following immersion in the dip, so that the figures for engorged ticks given in Table 14 might legitimately be amended to read: 1061, 34 and 6 or, on a percentage basis: 100, 3·2, and 0·6.

When these results are considered in conjunction with Heath's (1946) finding that females which succeed in engorging on sheep which have been dipped in D.D.T. do not appear to survive to lay eggs, it is reasonable to assume that 3 dippings in M 37 prevented any female from successfully engorging during the spring tick season.

(*k*) *B.H.C. as an anti-tick dip*

Downing (1947) showed that D.D.T. is very much inferior to B.H.C. when used against psoroptic mange, and this made it desirable to investigate the possibility of using B.H.C. as an acaricide. Burtt (1945) showed B.H.C. to be toxic to ticks in laboratory experiments, and a series of field trials by the original method of testing tick dips indicated that, at a

Table 13. *D.D.T. as an anti-tick dip. Mean tick counts*

	Days after dipping																
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	87
Undipped	0·8	5·3	16·0	20·8	14·7	17·2	26·0	48·7	17·3	45·7	23·5	14·3	7·4	13·8	12·2	3·8	0·2
Commercial dip	0·0	0·3	6·0	1·2	1·6	0·5	2·0	6·8	18·7	0·0	1·8	0·0	0·5	5·7	5·7	0·0	0·0
D.D.T.	0·0	0·0	0·0	0·3	0·0	0·0	0·2	0·3	0·0	0·7	0·0	0·2	0·0	0·0	0·0	0·0	0·0

Table 14. *D.D.T. as an anti-tick dip*

	Undipped	Commercial dip	D.D.T.
No. of ticks which attached	1385	519	49
No. of ticks which detached before engorging	124	167	20
No. of ticks which died *in situ*	200	284	21
No. of ticks which engorged	1061	68	8

When these results are considered in conjunction with Heath's (1946) finding that females which succeed in engorging on sheep which have been dipped in D.D.T. do not appear to survive to lay eggs, it is reasonable to assume that 3 dippings in M 37 prevented any female from successfully engorging during the spring tick season.

similar concentration, B.H.C. with a γ-isomer content of about 12% is roughly as effective as D.D.T. when used in similar emulsions, but that the protective effect begins to disappear about 5 days earlier than in sheep which have been dipped in D.D.T.

In 1946, a commercial anti-tick dip containing B.H.C. was already on the market. A field trial of this dip against M 34 (Heath & Mitchell, 1946) at bath concentrations of 0·5 and 0·25% D.D.T. and M 37 at bath concentrations of 0·25 and 0·1% D.D.T. gave results which are summarized in Table 15. The commercial dip was of little value.

To compare B.H.C. and D.D.T. when in similar emulsions and concentrations, B.H.C. was incorporated in a miscible oil which was an exact copy of M 37 except for the fact that D.D.T. was omitted; the dip was called H 1. The following groups of six sheep were used:

Group 1. Undipped.

Group 2. Dipped in H 1 at bath conc. 0·05% B.H.C.

Group 3. Dipped in H 1 at bath conc. 0·25% B.H.C.

Group 4. Dipped in M 37 at bath conc. 0·05% D.D.T.

Group 5. Dipped in M 37 at bath conc. 0·25% D.D.T.

Results, summarized in Table 16, indicate that B.H.C. is approximately as effective as D.D.T. when used in similar emulsions at the same bath concentration. It is assumed that the γ-isomer of B.H.C. is the most actively acaricidal part of the compound (although this point has not yet been fully investigated in the case of *I. ricinus*) and the B.H.C. used in H 1 contained approximately 16% γ-isomer. Therefore, it may be tentatively stated that the γ-isomer of B.H.C. is approximately as effective as D.D.T. against the sheep tick when used at a dilution 5 times greater.

In 1947, another commercial anti-tick dip containing B.H.C. was tried out in the field. The following groups of sheep were used:

Group 1. Undipped.

Group 2. Dipped in the commercial dip. Results of the initial dipping were so disappointing that this group was redipped at 1·33 times the recommended strength 11 days afterwards. The redipping also gave disappointing results, so the group was redipped at 2·66 times the recommended strength 13 days after the second dipping.

Group 3. Dipped in M 37 at a bath concentration of 0·5% D.D.T. Tick watching continued for 41 days after the initial dip. When considering the results of this trial (Table 17), it is necessary to remember that sheep in group 2 were dipped twice after the initial dipping, which means that, on two occasions, the entire infestation was wiped out and the group got away to a fresh start. The dip is of little value as a means of tick control.

Table 15. *Efficacy of a commercial anti-dick dip containing benzene hexachloride*

			M 34		M 37	
	Undipped	Commercial B.H.C. dip	0·5%	0·25%	0·25%	0·1%
No. of ticks which attached	423	288	113	133	33	58
No. of ticks which detached before engorging	50	39	21	21	6	9
No. of ticks which died *in situ*	42	24	24	23	16	20
No. of ticks which engorged	246	171	47	62	5	10

Table 16. *Comparison of D.D.T. and B.H.C. as anti-tick dips.*

		H 1		M 37	
	Undipped	0·25%	0·05%	0·25%	0·05%
No. of ticks which attached	269	36	79	28	55
No. of ticks which detached before engorging	39	3	13	6	4
No. of ticks which died *in situ*	26	26	19	13	12
No. of ticks which engorged	159	5	35	4	11

(*l*) *An improved method of applying acaricides*

It has been shown above that almost the entire female tick population on a sheep is to be found on the head, axillae and inguinal region, and that the small number which attach in the fleece probably do not commonly escape after engorging. Many attempts have been made to evolve a method of treating these sites, and the following method appears to be satisfactory. The sheep are walked through a shallow bath of dip, the heads being immersed twice during the process; the level of the dip is constantly adjusted to keep the bath 'belly deep'. Trials have shown that this method gives results which are as good as those achieved by complete immersion, and it has the advantage that: (*a*) it uses only about one-quarter as much dip, (*b*) danger of percutaneous absorption is lessened, and (*c*) sheep are handled much more rapidly.

(*m*) *Treatment of pastures with acaricides*

By means of a Todd Insecticidal Fog Applicator, large areas of tick-infested pasture were treated with D.D.T. in the form of a dispersible powder, a miscible oil, and a solution. It is regretted that exact details of areas treated and amount of D.D.T. applied per acre cannot be stated, because, although the work was done in fairly calm weather, a large proportion of the insecticidal fog drifted off the pasture at which it was aimed. However, even on areas which were known to have been heavily treated, blanket counts showed no difference in the tick population before and after treatment. Smith & Gouck (1945) successfully controlled *I. ricinus scapularis* (Say) by spraying roadsides with D.D.T. emulsions.

(*n*) *Acaricides* per os

Lindquist, Madden & Watts (1944) showed that adult bed bugs, *Cimex lectularis*, are killed when allowed to feed on rabbits which have previously been dosed with 228–537 mg. D.D.T. A field trial of D.D.T. *per os* as a means of killing ticks on sheep gave negative results. Two sheep received a total of 17·9 g. D.D.T. each over a period of 63 days (highest single dose was 0·9 g.) and two sheep each received a total of 31·5 g. D.D.T. during the same period (maximum dose 1·8 g.). The D.D.T. was administered in solution in arachis oil. Thirty-two engorged females and 503 engorged nymphs were collected from the four dosed sheep during the course of the trial, and twenty-six engorged females and 434 engorged nymphs were collected from four control sheep during the same period. Dr J. A. Campbell, Department of Zoology, Edinburgh University, kindly incubated these ticks and reported that there was no significant difference between the survival rates of ticks collected from the various groups. Table 18 shows mean tick counts on dosed and control sheep.

Wilson (1947) protected cattle from attack by ticks (*Rhipecephalus appendiculatus*, *R. evertsi*, *Amblyoma variegatum* and *Palpoboophilus decoloratus*) by dosing them with B.H.C. The following groups of sheep were used to investigate the possibility of controlling *Ixodes ricinus* by this method:

Group 1. Two untreated.

Group 2. Two which received daily doses of 50 mg. B.H.C. each.

Group 3. Two which received daily doses of 0·5 g. B.H.C. for 10 days, followed by 0·75 g. daily for 12 days, and 1·0 g. daily during the next 8 days.

Group 4. Two which received a weekly dose of 5 g. B.H.C. The average weight of these sheep was about 80 lb. One sheep in each of the dosed groups received its B.H.C. in the form of a 1·0 % (group 2) or a 2·5 % (groups 3 and 4) solution in arachis oil; the remaining sheep were dosed with B.H.C. in gelatin capsules. There appeared to be no difference between the effects of the two methods of dosing. Engorged females were collected from all sheep, tubed individually, and kept in a common atmosphere at 100 % relative humidity. Table 19 shows results of intensive tick watching on these sheep, and Table 20 summarizes the survival rates of ticks collected. The treatment appears to have little promise as a method of tick control, although the survival rate in ticks was significantly lower in group 3 (by χ-squared test) than in those taken from control sheep.

Table 17. *Efficacy of a commercial anti-tick dip containing benzene hexachloride*

	Undipped	Commercial B.H.C. dip	M37
No. of ticks which attached	692	220	171
No. of ticks which detached before engorging	77	47	27
No. of ticks which died *in situ*	101	61	46
No. of ticks which engorged	375	62	52

Table 18. *Attachment of ticks to sheep which have been dosed with D.D.T. Mean tick count*

	Days after beginning dosing												
	1	6	11	16	21	26	31	36	41	46	51	56	63
Untreated	9·3	12·3	9·3	4·0	17·5	19·3	11·0	3·3	1·5	0·5	0·0	0·3	2·5
Dosed	13·3	23·0	14·8	9·8	26·0	26·7	18·3	3·5	2·3	0·8	1·5	0·3	0·3

(*o*) *Eradication of ticks from a pasture by dipping sheep*

Application of improved methods of control. Milne (1947*a*) suggests that up to 98 % of the female tick population is fed by sheep, when cattle, deer and hares are absent from the pasture. Heath (1946) points out that a mortality rate between egg and adult of up to 99·8 % must exist in areas where the tick population is static. Consideration of these facts suggests that, by the use of D.D.T. or B.H.C. dips, it might be possible to eradicate the tick population from an area grazed mainly by sheep. Indeed, Milne

(1948) stated: 'Since the sheep flock supports almost all the female ticks on a pasture, control is most likely to follow the development of an efficient acaricidal sheep dip'. Macleod (1934) and Moore (1938*a*, *b*, 1939, 1942) investigated the possibility of total eradication of the tick from selected areas. Their methods and results have been ably discussed by Milne (1949). In this work, during the winter of 1946–7, an area of approximately 12 acres adjoining open fell was enclosed on a farm in Cumberland, sub-enclosures of approximately ¼ acre each being fenced off in the south-east and south-west corners. Owing to technical difficulties, tick population of the area was not estimated during previous years, but it is known that sheep which grazed it in previous years acquired heavy tick infestations. It was planned to run nothing but sheep dipped in D.D.T. in the main enclosure, leave the subenclosures untenanted, and use the area outside the fence as the control, since sheep treated according to normal farm practices graze this area. A dipping tank was installed in a corner of the main enclosure so that, when necessary, sheep within the enclosure could be redipped without being driven across tick-infected ground. Since 11 April 1947, dipped sheep in numbers ranging from four to sixty have been kept in the main enclosure. Owing to delay in obtaining supplies, it was not possible to use the same dip throughout, but all sheep were dipped before entering the enclosure. Sheep already in the enclosure were dipped as often as was necessary to prevent any female tick from completing engorgement. Table 21 is a list of dates of dipping and type of dip used. Tick populations have been estimated by means of Campbell's 'static' blanketing method, results being summarized in Table 23. On 11 September 1948, three attached females and, on 28 September 1948, one attached female were found on the six sheep which were grazing the pasture; these ticks appeared to be healthy, and were picked off by hand and killed. With the exception of the four just mentioned, no ticks of any stage have been found attached to sheep within the main enclosure since autumn, 1947. Numbers of sheep which grazed the pasture are shown in Table 22. On 4 May 1949, one undipped lamb was found to have gained access to the paddock; it was immediately removed.

The results shown in Table 23 are unexpectedly encouraging because, to reach the enclosure, it is necessary to walk through about half a mile of tick-infested pasture, and it is almost inevitable that some unattached ticks are carried in on the clothes of the operator. Also, despite precautions, it would be possible for the sheep dogs to drop engorged ticks on the enclosure.

Table 19. *Attachment of ticks to sheep which have been dosed with benzene hexachloride*

	Group 1	Group 2	Group 3	Group 4
No. of ticks which attached	249	433	320	305
No. of ticks which detached before engorging	31	33	28	41
No. of ticks which died *in situ*	42	48	36	45
No. of ticks which engorged	132	271	200	158

Table 20. *Survival rates of ticks which engorged on sheep which had been dosed with benzene hexachloride*

	Undosed	Group 2	Group 3	Group 4
No. of females collected	39	50	27	14
No. which oviposited	36	37	15	10
No. which died before ovipositing	3	13	12	4
Percentage survival rate	92·3	74·0	55·6	71·4

Table 21. *Record of dippings in tick-eradication trial*

Date	Dip used
11 Apr. 1947	Macleod's arsenic-derris dip (sheep had been dipped in M 37 9 days previously)
25 Apr. 1947	M 37 at bath concn. 0·5 % D.D.T.
20 May 1947	M 37 at bath concn. 0·5 % D.D.T.
4 June 1947	M 37 at bath concn. 0·5 % D.D.T.
23 July 1947	M 37 at bath concn. 0·5 % D.D.T.
5 Sept. 1947	M 37 at bath concn. 0·5 % D.D.T.
17 Oct. 1947	M 37 at bath concn. 0·5 % D.D.T.
17 Apr. 1948	H 1 at bath concn. 0·5 % B.H.C.
18 Aug. 1948	M 37 at bath concn. 0·5 % D.D.T.

Table 22. *Numbers of dipped sheep kept in pasture during tick-eradication attempt*

Date	Sheep in pasture
11 Apr. 1947	6 hoggs; remained in until 15 Oct. 1948
17 Oct. 1947	66 adults; remained in until 21 Oct. 1947
Apr. 1948	4 lambs born in pasture; remained until 27 Oct. 1949
4 May 1949	1 undipped lamb broke into the pasture; removed same day
3 May 1949	18 ewes and 16 lambs; remained until 28 June 1949
25 Nov. 1949	60 adults; remained until 18 Dec. 1949

SUMMARY AND CONCLUSIONS

Because of its ability to carry diseases, the sheep tick should be classed as one of the most important ectoparasites of British sheep. No reason for the differences between individual susceptibility to infestation has been found.

Commercial arsenical dips are excellent acaricides, but give little protection from reinfestation, and are no better than watery solutions of sodium arsenite at the same concentrations of arsenic. In the work reported, addition of derris to the commercial dip did not improve it.

Coal-tar fractions which were tested were of little value in tick control, and five finely divided inert powders were not acaricidal.

D.D.T. and B.H.C. give promise of being of real value in controlling ticks; since the former is not effective against sheep scab, it is suggested that future research on anti-tick dips be confined to preparations containing B.H.C., the γ content of which is probably about as effective as D.D.T. against ticks when used at a 5 times greater dilution. Two commercial dips containing B.H.C. were found to be of little value.

A method of anti-tick treatment which involves walking sheep through a shallow bath of dip holds great promise.

Treatment of pastures with D.D.T. appeared to have no effect upon the tick population, and neither D.D.T. nor B.H.C. was effective against ticks when administered to sheep *per os*.

By stocking a pasture with sheep which were dipped sufficiently often to prevent any female tick from engorging, the tick population was almost completely eradicated in one year. Sheep which were born in the pasture in April 1948, and remained there until October 1949, were not dipped during 1949 and, as far as is known, were not at any time infested by ticks. Pastures from which all sheep were excluded gradually lost their tick populations, but a few ticks remained at the end of 3 years. This may be because, in the main enclosure, almost every tick would have an opportunity of attaching on a dipped sheep, and would be killed; in the unstocked enclosures, lack of opportunity to attach on sheep would result in a larger number of ticks infesting wild animals and so a few ticks would be carried over from year to year. This result is unlikely to have any practical application at present, because ridding a small area (e.g. a farm or a valley) of ticks would result in the sheep of that area losing their immunity to tick-borne diseases, and re-introduction of ticks might bring disastrous results. Also, it should be pointed out that work quoted in the text gives the result of an attempt at eradication in one county of Britain. Among other things, the fact that cattle, hare, and deer were absent immediately prohibits application of the results to many other areas of Great Britain. However, it appears that, in the absence of hare and deer, it might be possible to rid an area of ticks by adequate treatment of domestic animals.

Table 23. *Number of ticks recovered on 'static' blankets**

	Dipped enclosure				Starved enclosure (A)				Starved enclosure (B)				Control area			
	♀♀	♂♂	oo	LL	♀♀	♂♂	oo	LL	♀♀	♂♂	oo	LL	♀♀	♂♂	oo	LL
Spring 1947	10	11	126	5	17	15	103	13	10	8	68	4	7	7	98	3
Autumn 1947	8	5	63	0	56	41	295	1	26	15	364	2	6	5	201	4
Spring 1948	0	0	1	0	5	4	45	7	0	1	62	0	3	4	251	12
Autumn 1948	0	0	1	0	1	3	65	1	0	0	125	0	0	0	56	2
Spring 1949	0	0	0	0	1	0	8	0	0	0	4	0	6	10	146	7
Autumn 1949	0	0	0	0	0	0	5	0	0	0	4	0	3	4	16	52

* Number of blankets examined in *each* enclosure was: spring 1947, 99; autumn 1947, 134; spring 1948, 117; autumn 1948, 57; spring 1949, 63; autumn 1949, 69.

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APPENDIX 1

Amount of blood withdrawn from sheep by ticks

Clean white linen sacks, each containing larvae from one female tick, were attached around the depilated tail of a sheep. After the larvae had engorged and dropped into the bag, their iron content was estimated by the Weybridge modification of the method described by Breur & Militzer (1938). From this, the amount of haemoglobin and then blood was calculated. As a cross-check on this calculation, samples of blood were withdrawn from the jugular veins of infested sheep, and the amount of blood withdrawn by ticks was calculated as a direct proportion to the iron content of 0·2 ml. sheep's blood. Results of the two methods of calculation were in close agreement. Tick faeces from the bag were examined similarly. Debris from control sacks containing no larvae, and left on the sheep's tail for several days contained no iron. Faeces from nymphs and females have not been collected, but the blood content of engorged nymphs and females was calculated from their iron contents. Results are summarized in Table 24.

Table 24

	Mean blood content (ml.)
Unfed larva (20 estimations on batches of 100)	0·00006
Engorged larva (8 estimations on batches of 100)	0·00162
Faeces from 1 larva (calculated from total from 800 larvae)	0·00252
Engorged nymph (15 estimations on batches of 10)	0·01054
Engorged female before oviposition (20 estimations)	0·45000
Engorged female after oviposition (4 estimations)	0·44800

During the spring tick season of 1947, 1385 female ticks attached to the face, ears and axillae of six sheep running on moderately heavily tick-infested ground in Cumberland. Heath & Mitchell (1946) showed that this figure represents approximately 87 % of the total ticks on the whole body, if the small numbers which attach in the fleece be disregarded. Thus, the above figure may be corrected to give an estimated *total* of 1591 ticks on the six sheep, or 265 ticks per sheep. A proportion (23 %) of these ticks either detached before engorgement was complete or died during the process of engorging but, for purposes of calculating blood loss, it may be assumed that they each removed 0·45 ml. of blood (Table 24) plus the amount passed through as faeces. Lees (1946) found that the female tick ingests about 600 mg. of blood which, in a healthy sheep, would represent a volume of approximately 0·57 ml. No information about the amount of blood passed in the faeces of nymphs is available, but, for purposes of calculation, the ratio of blood retained : total blood removed might be placed midway between that of females (1·0 : 1·27) and of larvae (1·0 : 2·555), to give a ratio of 1·0 : 1·913.

Milne (1947*b*) estimates that the ratio of nymphs to females fed by Cheviot sheep is approximately 8·5 : 1·0 so that, if this ratio holds for Cumbrian sheep, infestation by nymphs during the 1947 spring tick season must have been in the neighbourhood of 2253 per sheep.

No figures are available as to the number of larvae which feed on sheep, but it might be reasonable to assume that 20 % of the eggs either fail to hatch, or the resulting larvae fail to find a host. Macleod (1933) estimates that each female lays 500–2000 eggs, and 1200 eggs per female may be taken as a fair average number. The arbitrary assumption, for purposes of calculation, of a 20 % mortality among larvae would thus leave a ratio of larvae : nymphs : females engorging on sheep :: 960 : 8·5 : 1, and the blood loss per sheep due to tick infestation of the six sheep cited above, during the spring tick season of 1947, may be estimated as being:

Blood removed by	ml.
265 females	152
265 × 8·5 nymphs	45
265 × 960 larvae	1052
Total blood loss due to ticks	1250

No high degree of accuracy can be claimed for this calculation, but it probably approximates the truth. The loss of blood was spread over the period roughly 15 March to 30 June, and it is unlikely that removal of blood at this rate would significantly influence the condition of the sheep.

APPENDIX 2

Trials of coal-tar fractions as anti-tick dips

Active constituent(s)	Concn. (%)	Emulsifier	Remarks on field test
Low temp. tar phenols, b.p. 240–250° C.	0·5	Sodium oleate	So painful to sheep that dip discarded
Low temp. tar phenols, decolorized	0·5	Sodium oleate	
Low temp. tar phenols, b.p. 250–260° C.	0·37	Sodium oleate	
Low temp. tar phenols, b.p. 250–260° C.	0·5	Sodium oleate	
Low temp. tar phenols, b.p. 220–250° C.	0·5	Casein in ammonia and Teepol X	
Lupetidine, b.p. 220–250° C.	0·4		
Low temp. tar phenols, b.p. 230–240° C.	0·1	Sodium oleate	Not acaricidal or repellent
Low temp. tar phenols, b.p. 230–240° C.	0·2	Sodium oleate	
Pentachlorphenol in cresylic acid	0·1	Sodium oleate	
Sodium pentachlorphenate	0·5	Sodium oleate	
Low temp. tar cresylic acid, b.p. 180–210° C.	0·5	Resin soap	
High temp. tar cresylic acid, b.p. 180–210° C.	0·5	Resin soap	
Low temp. tar xylenols, b.p. 210–220° C.	0·5	Resin soap	
Homologues of pyridine, 90% distilling below 180° C.	0·5	Casein in ammonia	
Homologues of pyridine, 90% distilling below 200° C.	0·5	Casein in ammonia	
Lauryl thiocyanate	0·05	Teepol X	
Chlorinated cresylic acid (50% Cl)	0·35	Sodium oleate	Slightly acaricidal, but not repellent
Chlorinated xylenol (30% Cl)	0·3	Sodium oleate	
Chlorinated low temp. 'XL' tar acids (5% Cl)	0·5	Sodium oleate	
Low temp. tar phenols, b.p. 230–240° C., decolorized	0·5	Sodium oleate	
Low temp. tar, phenols, b.p. 220–250° C.	0·5	Sodium oleate	
Vertical retort tar phenols, b.p. 250–300° C., decolorized	0·5	Sodium oleate	
Vertical retort tar phenols, b.p. 250–300° C., purified from neutral oil	0·5	Sodium oleate	
High temp. tar oil, b.p. 280–360° C. after removal of phenols	1·0	Casein in ammonia and resin soap	
Low temp. tar oil, b.p. 300–360° C.	1·0	Casein in ammonia and resin soap	
Low temp. tar oil, b.p. 300–360° C. after removal of phenols	1·0	Casein in ammonia and resin soap	
Coal tar bases, b.p. 130–250° C.	0·5	Casein in ammonia and resin soap	
Coal tar bases, b.p. 220–275° C.	0·5	Casein in ammonia and resin soap	
High temp. tar oil, b.p. 280–360° C.	1·0	Casein in ammonia and resin soap	
Low temp. tar cresylic acid, b.p. 180–210° C.	0·5	Resin soap	
High temp. tar cresylic acid, b.p. 180–210° C.	1·0	Resin soap	
Low temp. tar xylenols, b.p. 210–220° C.	1·0	Resin soap	
Shell-Mex spindle oil	1·0	Resin soap	
Low temp. tar phenols, b.p. 220–250° C.	0·5	Sodium salt of wool grease acids	
Nicotine base	0·5		
Low temp. tar phenols, b.p. 220–250° C., decolorized	0·5	Sodium salt of wool grease acids	
Neutral wool grease	2·0	Sodium salt of wool grease acids	
Low temp. tar phenols, b.p. 220–250° C., decolorized	0·5	Sodium salt of wool grease acids	
Vertical retort tar phenols, b.p. 220–250° C., decolorized	0·7	Sodium oleate	Slightly acaricidal, not repellent, painful to sheep
Low temp. tar phenols, b.p. 220–250° C., decolorized	0·5	Sodium oleate	
Low temp. tar phenols, b.p. 220–250° C.	0·5	Casein in ammonia	
Low temp. tar. phenols, aniline	0·35	Casein in ammonia	

Active constituent(s)	Concn. (%)	Emulsifier	Remarks on field test
Chlorinated low temp. tar acids (30% Cl.), b.p. 220–230° C.	0·5	Sodium oleate	All sheep became ill and died within 15 min. of leaving bath
Low temp. tar phenols, b.p. 220–250° C. Lutidine fraction	0·5 0·35	Casein in ammonia	
Vertical retort tar phenols, b.p. 220–280° C., decolorized and purified from neutral oil.	0·7	Sodium oleate	Good acaricide, not repellent, painful to sheep
Low temp. tar phenols, b.p. 300° C.	0·7	Sodium oleate	Slightly acaricidal and repellent
Low temp. 'XL' tar acids, b.p. 220–270° C.	0·5	Sodium oleate	Slightly acaricidial and repellent, all sheep became ill and died within 2 days of dipping
Low temp. tar phenols, b.p. 230–240° C.	0·5	Sodium oleate	
Cresylic acid Rotenone	0·2 0·02	Sodium oleate	Poor acaricide; repellency equal to commercial dip
Low temp. tar phenols, b.p. 220–250° C.	0·5	Sodium oleate	Moderately acaricidal; not repellent
Chlorinated low temp. 'XL' tar acids (10% Cl)	0·5	Sodium oleate	Moderately acaricidal; slightly repellent
Low temp. tar phenols, b.p. 220–250° C.	1·0	Sodium oleate	Good acaricide; slightly repellent
Low temp. tar phenols, b.p. 250–300° C.	0·7	Sodium oleate	Good acaricide; slightly repellent; painful to sheep
Low temp. tar phenols, b.p. 250–300° C.	1·0	Sodium oleate	Equal to commercial dip; 'Scalded' axillae
Chlorinated low temp. 'XL' tar acids (15% Cl)	0·5	Sodium oleate	Equal to commercial dip
Low temp. tar acids, b.p. 220–250° C., decolorized Rotenone	0·2 0·02	Sodium oleate	
Low temp. tar phenols, b.p. 220–250° C. decolorized	0·5	Sodium salt of wool grease acids	
Sodium arsenite	0·22	Sodium salt of wool grease acids	
Neutral wool grease	2·0	Sodium salt of wool grease acids	
Sodium arsenite	0·25	Sodium salt of wool grease acids	
Sodium arsenite	0·22	None	

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IMMUNITY AND METABOLIC RATE: THE COURSE OF NOTOEDRIC SCABIES IN RATS WITH DIFFERENT METABOLIC RATES

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(With 2 Figures in the Text)

Preliminary studies of epidemics of rat scabies showed that thyroidal treatment had marked effects on the course of the disease; the experiments reported here were designed to extend these observations by a statistical study of the differences between epidemics of notoedric scabies on rats at normal, raised, or lowered metabolic rates.

A brief summary of the biology of the mite and of its association with disease in the rat is included in explanation of the experimental procedure and findings.

BIOLOGY OF THE PARASITE

Scabies of rats is the result of infestation with the Sarcoptid mite, *Notoedres muris* Megnin, the biology of which is similar to that of *Sarcoptes scabiei*, the cause of scabies in man and other mammals.

The life history of *Notoedres* was first investigated by Gordon, Unsworth & Seaton (1943). *Notoedres* is a burrowing mite; the females tunnel in the deeper layers of the skin where each may live 3–4 weeks laying about sixty eggs. The larvae hatch out in 4–5 days and leave their maternal burrows for the skin surface where they may wander for some hours. These larvae make shallow pockets in the skin in which they moult about the 10th day after oviposition. The first-stage nympha which emerges from this moult makes a similar pocket in which it in turn moults on the 13th day. This second nympha also makes a shallow pocket and moults again, giving rise to the male or the ovigerous female about the 16th day. The adult female extends her moulting pocket to form the deep 'nesting' tunnel in which, after a few days, she begins to lay her eggs, thus starting a new cycle.

The disease is spread over the body by the dispersal of larvae and nymphae, clean animals being infected by contact transference. A prepatent period of some 10 days may elapse before the first obvious signs of the nesting tunnels can be seen on infected animals.

Lesions produced by the parasite

The preparation of the tunnel provokes an inflammatory reaction visible externally as a zone of reddened skin surrounding a plug of coagulated exudate. These papules are the only lesions easily visible to the naked eye—the shallow moulting pockets cannot be seen without the aid of a microscope. Each tunnel persists throughout the life of the female mite within it; when she dies the lesion heals within a week. The individual lesions are easily discerned but as the disease progresses groups of burrows may be covered over with continuous encrustation.

Lesions are generally confined to the naked or sparsely vested areas of the body, particularly the skin of the tail, ears, nose, feet and scrotum. On severely affected animals the disease may spread to the hairy parts, especially the sacral region.

METHODS AND MATERIAL

The host animals were male white rats from a line-bred strain; they were first infected as young adults weighing about 200 g. and were kept in communities of five in wire cages. They were fed on a nutritionally adequate diet in meal form mixed with its own weight of milk. Twice weekly they received as much fresh liver or heart as they would eat.

The disease was transmitted to the experimental rats by introducing one infected rat to each cage. These infecting rats were of even clinical appearance, they were changed from cage to cage each day to ensure an even opportunity of transference and they were removed after 2 weeks. The course of the disease was followed by counting on each rat at weekly intervals the number of tunnels; these counts were accurate in the early stages; later, when groups of lesions became hidden under a single scab the number of females in the groups were estimated on the basis of dissections of similar lesions in the rats not included in the experiment. The counts on the tail, ears, nose, feet, scrotum and other sites were recorded separately.

Iodinated casein (Reineke, Williamson & Turner, 1943) was used to elevate basal metabolism and 4-methyl-2-thiouracil to depress it. On hydrolysis of iodinated casein, *l*-thyroxine is liberated (Ludwig & von Mutzenbecher, 1939). Assays on the tadpoles of *Xenopus laevis* (Deanesly & Parkes, 1945*a*) showed that the preparation used was approximately equal in potency to the reference preparation

* Statistical and physiological work.
† Parasitological work.

NC 4+5 (Deansley & Parkes, 1945*b*), 1 g. of which has been shown to be equivalent to approximately 4·7 mg. *l*-thyroxine (Bailey, Bartlett & Folley, 1949). The rats ate about 25 g. of the dry diet daily. Incorporation of 0·8 g./kg. of iodinated casein allowed an average daily intake of 20 mg. of the drug approximately equivalent to 0·1 mg. *l*-thyroxine/kg. body weight.

4-Methyl-2-thiouracil was mixed with the dry diet at the rate of 0·4 g./kg. of meal, giving a daily intake of approximately 50 mg./kg. body weight. Reineke, Mixner & Turner (1945) have shown that 2-thiouracil, a drug of equivalent goitrogenic potency (Astwood, Bissell & Hughes, 1945), depressed the metabolic rate of rats by 24% when supplied in the drinking water at a dilution of 1 : 1000.

Thirty rats were arranged in six cages so that the total weights of the five rats in each cage were as nearly equal as possible. Two cages were allocated at random to each treatment.

Three treatments were given in both experiments:

(1) Iodinated casein, 0·8 g./kg. of dry diet.
(2) Methyl thiouracil, 0·4 g./kg. of dry diet.
(3) Untreated controls.

RESULTS

Experiment 1. *The effect of the level of metabolism on resistance to infestation*

Three groups of ten rats were treated as described above for 3 weeks before being infected with *Notoedres*. Lesions were first counted on the 3rd week after exposure to infection, by which time a generation of mature female mites had developed. For the next 19 weeks the course of the disease was recorded by weekly counts of lesions, plotted in Fig. 1.

In the untreated controls, the peak of the epidemic, with an average maximum infestation of 263 mites per rat, occurred between the 6th and 8th weeks after infection had been introduced. The effect of thiouracil was to delay the onset of recovery, so that the rise in mite counts continued until the 11th week, reaching a peak of 358 mites per rat, well above that of the controls. Even at the end of the 20th week, recovery was less complete than in the control group. The rate of increase in the group receiving iodinated casein was very much slower; there was no sharp peak to the epidemic and the maximum population reached was only eighty-eight mites per rat. Even at the end of 20 weeks of treatment the mite population was still much smaller than that of the controls.

Table 1. *Mean total count of lesions per rat*

Treatment	Mean count	Standard error
Thiouracil	4248	±761
Control	2924	±675
Iodinated casein	1184	±233

The treatment also affected the appearance and fate of individual lesions. In the hyperthyroid group, for example, the local reaction to burrowing

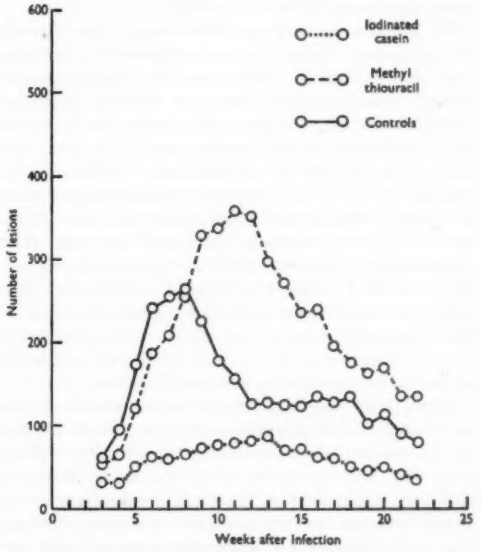

Fig. 1. Weekly counts of scabies lesions: average of ten rats. Metabolism treatment began 3 weeks before exposure to infection and first counts were made 3 weeks after exposure.

by the mites was intense, each tunnel being surrounded by a zone more acutely inflamed than the normal. There seemed to be more exudation in these burrows and greater encrustation. Serum casts of these burrows could often be dissected out intact,

Table 2. *Percentage of the total infestation in each site*

Treatment	Nose	Ears	Tail	Feet	Scrotum	General body surface	Total
Control	4·08	22·25	59·36	2·87	11·12	0·32	100
Iodinated casein	2·62	30·76	58·13	3·06	5·44	0·00	100
Thiouracil	4·32	22·74	49·68	7·81	12·17	3·28	100
Total	11·02	75·75	167·17	13·74	28·73	3·60	300

and in many of these the mite was found immured with her eggs. This was noted less frequently in the normal group and still less in the hypothyroid rats. The eggs in these plugs seemed to be prevented from hatching, and the female mites were unable to continue their excavations. This local reaction appeared to become more intense a few weeks after infection.

Using sums of all weekly counts on individual rats as a measure of the overall effects of the treatments, the results given in Table 1 show that the level of metabolism had marked effects on the total infestation.

For statistical analysis the data were transformed to logarithms, to equalize the variance in the three groups. Results showed that the effects of treatments were highly significant ($P < 0{\cdot}001$).

In addition to causing differences in the severity of the disease, the treatments affected the distribution of the lesions. The data on this point are presented as percentage figures in Table 2. Iodinated casein, while reducing the total population, increased the proportion on the ears ($+9{\cdot}27\,\% \pm 2{\cdot}45$) and reduced the proportions on the nose ($-1{\cdot}63\,\% \pm 0{\cdot}77$), scrotum ($-5{\cdot}15\,\% \pm 2{\cdot}02$) and general body surface ($-0{\cdot}34\,\%$). Thiouracil reduced the proportion on the tail ($-10{\cdot}02\,\% \pm 3{\cdot}42$) and increased the proportion on the feet ($+5{\cdot}04\,\% \pm 0{\cdot}75$) and general body surface ($+2{\cdot}81\,\%$). On the tails of the hyperthyroid rats, the lesions were evenly dispersed, whereas on the hypothyroid rats, the mites were grouped in a dense band around the root where the hair-cover presumably provided a warmer situation.

It may be concluded from this experiment that increase in basal metabolic rate protected the rats by increasing their resistance to scabies infestation. This had the effect of restricting the mites to the bare areas of the body. Conversely, the decreased metabolic rate reduced resistance allowing the disease to become much more severe, and permitting the mites to invade the hairy parts of the body.

Experiment 2. *Effect of metabolic rate on the recovery from infestation*

When it became clear that resistance to the development of rat scabies could be altered by changing the level of basal metabolism, it was felt that a modified experiment would give useful supplementary information. Accordingly, Exp. 2 was designed to show the effect of altering the metabolic rate when the parasitic infestation was already firmly established.

The only change from the plan and technique of the first experiment was that the rats were infected with *Notoedres* 25 days before the start of experimental feeding instead of 3 weeks afterwards. Lesions were counted each week, starting 14 days after infection was introduced. As in the previous experiment, the trend of the infestation from week to week can best be demonstrated in graph form (Fig. 2)

The course of the disease on the control rats was similar to that observed in the first experiment, and there were no differences between the three groups until the start of the experimental feeding. Treatment with iodinated casein, however, reduced the peak infestation from the normal of 285 lesions per rat to 235, and hastened the onset of recovery by 3 weeks. The infestation began to regress 4 weeks after the start of treatment, and eventually settled down at about seventy lesions per rat, half the number recorded on the controls. In the thiouracil

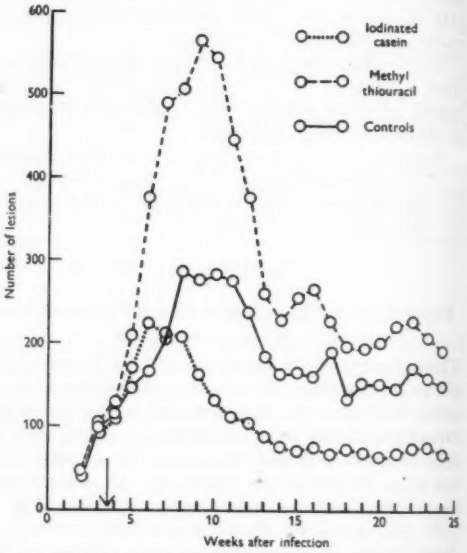

Fig. 2. Weekly count of scabies lesions: average of ten rats. The first counts were made 2 weeks after exposure to infection and the metabolism treatment began 3½ weeks after exposure (indicated by arrow).

group the infestation increased rapidly to twice that on the control rats: recovery began about the same time and exhibited a similar rate of regression to the controls, although the final level of parasitism remained about a third higher than that on the normal rats.

It may be concluded from this experiment that the course of the disease can be modified by alterations in basal metabolic rate made when the disease is already established.

Physiological effects of treatment with iodinated casein or thiouracil

Certain general effects of the treatments were noted in both experiments. In addition to the expected effects on weight gain, the three groups

had distinctly different clinical appearances. The elevated metabolism group had sleek, shining short soft pelage; they were constantly active, engaging in frequent play and reacting strongly to sudden noise and other stimuli; they ate more food, drank more water and soiled their cages sooner than other groups. By contrast, the group getting thiouracil seemed to be rather miserable and to feel the cold more; they grew much longer harsher coats with many bristly guard-hairs and even developed a covering over the scrotum which is normally bare. This group was inactive, dozed a lot and was flabby and toneless compared with the tenseness of the rats on the higher metabolic rate. The control (normal) group was intermediate in these respects. These differences became less marked as the experiment went on, for the animals appeared to develop some tolerance to the two drugs.

DISCUSSION

Some papers recording clinical observations on the relation of hyperthyroidism to disease in man, have been reviewed by Reitler (1947), and some relevant animal experiments have been reported by Larsh (1947) and Todd, Culton, Kelly & Hansen (1949). Larsh reported that when the basal metabolism of mice was lowered or raised there was a change in the rate of encystment of *Hymenolepsis* (Cestoda) larvae only in hosts made markedly hyperthyroid; in such mice there was a denser parasitization which Larsh attributed to reduced resistance following vitamin depletion of the hyperthyroid host. Todd and his colleagues found increased numbers of coccidial oocysts in the caecal faeces of a group of chicks made hyperthyroid by iodinated casein administration. Interpretation of both experiments was based on counts made at post-mortem examination of the hosts and it is thought that such differences, noted at the end of the experiments, are open to a variety of interpretations.

The course of a disease and the hosts' reaction doubtless reflect the number and activity of the invading agent. This relationship is usually traced by recording such indices as body temperature, serum antibodies or the rate of excretion of helminth eggs; the chain of reasoning connecting these indirect indices with the actual host-parasite relationship has, however, not always been thoroughly tested. On the other hand, in diseases caused by such external parasites as *Notoedres* this relationship presents an opportunity for continuous observation and the effects demonstrated in the present experiments are undoubtedly due to the treatments given and provide good evidence linking metabolic rate with resistance to *Notoedres* infestation.

Investigation of the immunological reaction was not part of the experiment, although incidental observations suggest possible explanations for its mechanism.

Mites were successful in invading the hairy parts of the body of the hypothyroid animals, whose skin temperature would be lower than normal, whereas on hyperthyroid rats they were confined to the bare, hairless extremities. This distribution indicates a sensitive temperature preference on the part of the mites. It is possible that strict temperature requirements restrict the mites to certain areas on the body and that the lowered skin temperatures of the hypothyroid animals offered a greater area suitable for colonization. This explanation seems inadequate, however, when the distribution on the tail is considered; on hypothyroid animals the infestation was concentrated in a narrow band around the base, whereas on normal and hyperthyroid rats the wide dispersal of lesions along the tail indicated a still greater area suitable for colonization.

The food intake may also play some indirect part in resistance to infestation, since the hyperthyroid rats ate more and the hypothyroid animals less food than the normal group. The diet, however, provided liberal amounts of the essential food constituents, and it is unlikely that any of the rats were suffering from dietary deficiencies. An aspect of this nutritional relation is recorded by Kartman (1943): while he stresses that the association is complex, he has shown that certain experimental avitaminoses permitted increase in the louse population of rats.

The intensity of the local inflammation provoked by the mite's burrowing seemed to determine the success of the immune reaction and the restraint of the epidemic; plentiful exudate which rapidly coagulates entraps many of the mites and completely immures them so that the host is protected against increase in the infestation. Mellanby (1944) records hypersensitization to reinfestation and marked local reaction to intradermal injection of mite extracts in human scabies subjects. The enhanced reaction observed in the present experiment after a few weeks' infection indicates that a similar phenomenon occurs in Notoedric scabies. This enhanced reaction after the sensitization period was most marked in the high metabolism group. In this connexion, it is of interest to note that Kallós & Müller (1932) observed intensified cutaneous reaction to intradermal injection of tuberculin in human subjects following administration of thyroxine. This suggests that it is by determining the intensity of the local allergic reaction that the level of the metabolism decides the course of the scabies epidemic.

Finally, it is of interest to observe from the figures that the mite population in each group was eventually reduced to a fairly constant level such as is reached in many parasitic infections when the

host and parasite have become adjusted to a balanced relation. In this experiment the tolerated residual population was lowest on the hyperthyroid rats: it seems likely therefore that the higher the level of metabolism the smaller is the stimulus necessary to provoke an adequate defence reaction against further increase of the parasite.

SUMMARY

1. Two experiments are described investigating the effects of level of metabolism on (*a*) resistance to, and (*b*) recovery from, Notoedric scabies of rats. The progress and severity of the disease was measured by weekly counts of the number of lesions; the metabolic rate in some rats was raised by administration of iodinated casein and in others was depressed by methyl thiouracil.

2. In normal rats, the initial severe phase of the disease was followed by a partial recovery with survival of a smaller population of parasites.

3. This recovery process in established infestations was hastened by elevation and retarded by depression of the metabolic rate.

4. Resistance to the development of severe disease was enhanced by elevation and reduced by depression of the basal metabolic rate.

5. The level of the residual infestation, after the severe phase of the disease had passed, was also affected by the treatment, being decreased by the higher but increased by the lower metabolic rate.

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THE GROWTH OF THE HARVEST MITE, *TROMBICULA AUTUMNALIS* SHAW

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(With 11 Figures in the Text)

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INTRODUCTION

The periodical revivals of interest in harvest mites during the last half century, and the fact that our knowledge of them was still very fragmentary, led one to suppose that they were a most elusive group to study. This conception is certainly valid if one considers the number of attempts that have been made to rear them experimentally in the laboratory. *Trombicula autumnalis*, our own British harvest mite, had evaded all attempts to rear it further than the nymphal stage, and in view of the permanent hypogean existence which the nymphs and adults lead at some considerable depth in the soil (Cockings, 1948) it is not surprising that difficulty has been encountered in trying to discover the food and environmental requirements of the post-larval stages.

A desire to include the harvest mite in a study of post-embryonic morphogenesis in the Acarina was primarily responsible for the attempt to devise a rearing method suitable for following closely the progressive change of form of individual mites as they advanced towards the realization of the adult stage, when kept in separate rearing chambers. Unfortunately, the methods adopted by previous workers—whether successful or otherwise—were not suitable, since the mites were reared in batches.

The present paper includes a description of a method, developed after many attempts over the past 3 years, which satisfied the above requirements. Although it is described here as a method for rearing harvest mites, it has been used, with appropriate feeding and humidity adjustments, for successfully rearing Tyroglyphid and Oribatid mites. The chief aim of the present work has been to investigate the growth and development of *T. autumnalis*, the British harvest mite, primarily of whole organisms, and the findings have been used as a basis for speculation on the life cycle in the natural environment.

REARING METHODS

The statement of Tanaka (1899) that *T. akamushi* was almost certainly connected with the transmission of 'scrub-typhus' gave to the harvest mites a medical importance which led to the first attempts by the Japanese to rear them through the life cycle. Miyajima & Okumura (1917) described the life cycle from the larva to the adult stage, but as Warburton (1928) remarked, the account lacked much needed detail. They fed the larvae on mice and reared a few nymphs to adults on a diet of pieces of melons, potatoes and other vegetables.

In this country and on the Continent, especially in France, numerous workers, including Hirst (1915), André (1920) and Keay (1937), have attempted to rear *T. autumnalis* but, with varying success, they were able to rear the mite only from the larva to the nymphal stage. Keay (1937) tried to rear the nymphs on the vegetable diet described by the Japanese but the attempts were unsuccessful.

The impact of 'scrub-typhus' upon men engaged in the Far East theatre of World War II revived an interest in the study of the life cycle of the harvest mite which led to the American workers Melvin (1946), Michener (1946) and Jenkins (1947) successfully maintaining a colony of *Eutrombicula batatas*. The larvae were fed on young chicks and the engorged larvae were transferred to a layer of sterile soil mixed with chicken manure, kept moist in a glass jar with water which seeped through a plaster of Paris base. The nymphs and adults were presumed to have fed on chicken manure and cannibalism was ignored. Jenkins (1947) placed infected box turtles (*Terrapene carolina*) as hosts upon the open mouth of a jar and engorged larvae dropped on to the layer of sterilized humus or sandy soil in which the nymphs and adults were reared on supplies of mosquito eggs, as recommended by Jayewickreme & Niles (1946).

However, as previously mentioned, the methods of earlier workers, successful or otherwise, for rearing harvest mites either in a layer of some suitable medium like sterile soil or in cotton-wool (Jayewickreme & Niles, 1947) were unsuitable for the purpose of observing the progressive growth of individuals and selecting appropriate stages as required for further study. In the harvest mite the developing stages of the larva, nymph, and adult resemble the pupal instar of an insect. The cyclic growth of a harvest mite therefore consists of pupa-like developmental phases which alternate with active growing phases when the mite accumulates reserves of food for further development, or as in the case of the adult as a prelude to maturity.

The larval host

The choice of a suitable laboratory host presented the first problem. Rabbits, guinea-pigs, mice and other mammals or birds are not good laboratory hosts because they require too much attention and feeding. Urination can be reduced by giving such hosts only a little water prior to their use but the engorged larvae, which are usually made to drop through a wire grid on to a collecting tray, are too often obscured by faeces and spilled food.

Rabbits, guinea-pigs, chicks and fully grown mice were rejected as hosts because they are very sensitive to the presence of the active mites on their bodies and their attentions accounted for an excessively high mortality of mites. This difficulty could of course be avoided by exposing the host to sites heavily infected with mites but all the other disadvantages described would follow.

Of the available laboratory animals young mice, about 8 days old or less, came closest to the requirements, viz. minimum attention with respect to feeding, little or no faeces and excreta, and sluggish movements. The young mice were easy to handle; they were fed either normally, or artificially with warm diluted cow's milk through a pipette at convenient intervals; and they required only a small space which allowed an arrangement of small dimensions to house them; it was therefore possible to use a Petri dish as a tray to trap the engorged larvae (Fig. 1).

Larval engorgement

Unfed larvae were collected from the surface of the soil either by using a simple light-trap (Jones, 1950*a*) or by simply collecting clusters of them, on favourable days, with a moist camel-hair brush. Pieces of filter-paper were placed on infected soil and left overnight or occasionally for a few days until the conditions were favourable for the aggregating habit to induce clusters to form on the frayed edges of the filter-paper.

The young mice were placed in closed glass dishes. A known number of mites, usually fifty, was transferred to a mouse with a dry camel-hair brush. The container was examined in about half an hour and larvae which had fallen into the dish were replaced upon the body of the mouse, but after an hour or so all the introduced mites had more or less securely attached themselves to the hairs of the host.

The infected mouse was placed upon a circular wire gauze (about 9·75 cm. in diameter) which rested, like a cover, on a Petri dish (9·25 cm. in diameter). An inverted glass funnel of appropriate size was placed upon the gauze and three upright metal strips clipped to the edge of the gauze kept the funnel in place (see Fig. 1). Young mice are sluggish and they were not perturbed by the mites and they soon settled in the dark. The mites showed a predilection for attachment around the anus as a distinct yellow cluster but they will attach themselves indiscriminately to other parts of the body. I have tentatively explained this habit of mites forming clusters on certain parts of the body, such as the anus and inside the ears, in a previous paper (Jones, 1950*d*). After about 36 hours the Petri dish was lined with a layer of moist cotton-wool which was covered with black filter-paper. The Petri dish was placed inside a large one, 12 cm. in diameter and the moat between their walls was filled with distilled water to prevent the escape of the very active fully engorged larvae after they have dropped off the host. At 25° C. the engorged larvae usually began dropping off the host after 48 hr. attachment, but some persisted for 3–4 days (see Table 1). The activity of the mouse when it was fed and exposed to the light of a lamp often induced considerable detachment of engorged larvae

The filter-paper rearing cell

The cell consisted of a Perspex framework (4 cm. square, and 0·3 cm. in thickness), with a central circular aperture having an inclined wall to give a diameter of 2 cm. on one side and 1·8 cm. on the other. A piece of filter-paper (about 3 cm. square) was pasted over the narrower diameter of the aperture. Another piece of black filter-paper (3·5–4 cm. square) was thoroughly wetted, and placed upon the opposite side, and gently but firmly pressed into the open end of the aperture. The resultant concave depression formed the floor of the actual chamber for housing the mites. A piece of moist filter-paper (3 cm. square) adhering to a cover-glass, was placed over the depressed cavity so that the enclosed chamber was completely lined with filter-paper (see Fig. 2).

A high relative humidity of about 95–100 % was maintained in the chamber by placing the cells upon glass rods embedded in a layer of moist cotton-wool in contact with distilled water, the whole arrangement being enclosed in a large sealed glass

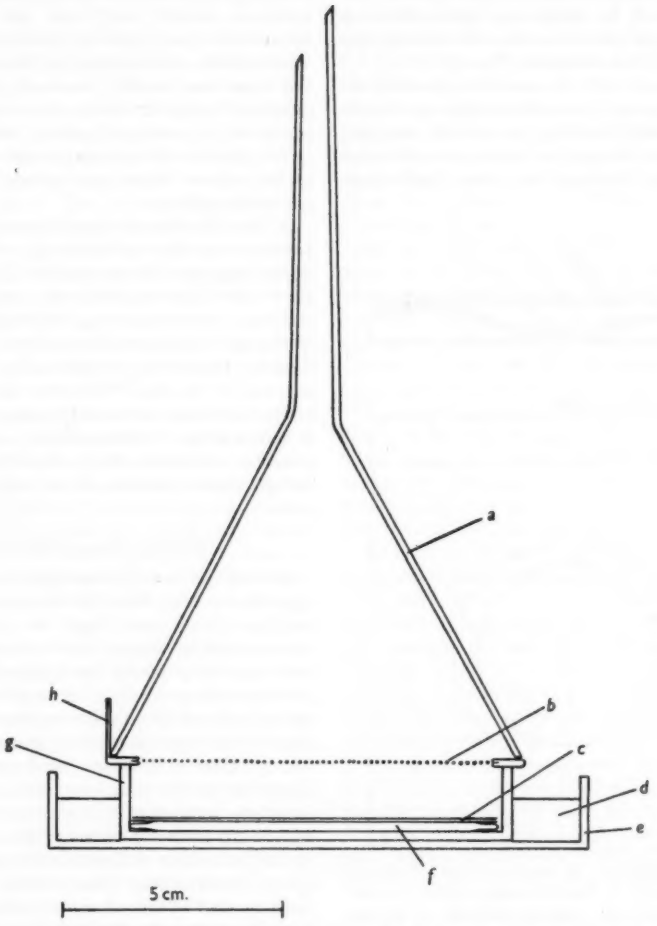

Fig. 1. Arrangement for confining host and recovering engorged larvae. *a*, glass funnel; *b*, wire grid; *c*, black filter-paper; *d*, distilled water; *e*, outer Petri dish; *f*, moist cotton-wool; *g*, inner Petri dish; *h*, clip.

Table 1. *Attachment period and recovery rate of engorged larvae of* Trombicula autumnalis *from host kept at* 24° C.

Batch	No. of larvae introduced on to two hosts	Duration and percentage recovery rate				
		1st day	24 hr.	48 hr.	72 hr.	4 days
1	100	introduction of larvae	—	52	67	—
2	100	introduction of larvae	—	37	49	—
3	100	introduction of larvae	—	33	58	72
4	100	introduction of larvae	—	25	42	—

trough. A small pad of cotton-wool was inserted between the filter-paper base and the floor of the chamber of the cell, to bridge any space between these surfaces and so allow the moisture to reach the filter-paper wall of the chamber (Fig. 2).

The cell eliminated all the careful and elaborate precautions taken by previous workers to choose suitable sterile culture media, to destroy moulds, and to prevent the formation of droplets of water which trapped and drowned the mites inside glass jars.

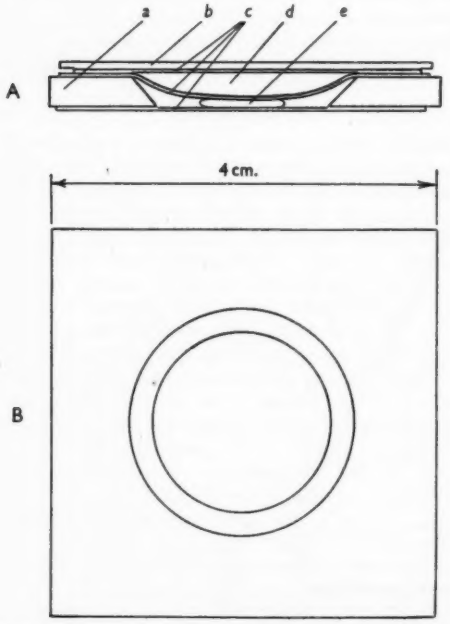

Fig. 2. The filter-paper cell. A, sectional view. B, plan of Perspex framework. *a*, Perspex framework; *b*, cover-glass; *c*, filter-paper; *d*, rearing chamber; *e*, cotton-wool plug.

The engorged larvae were very active and about twelve as a maximum were housed in the chamber of a cell. They were kept either at the laboratory temperature or at 25 or 30° C. as required, and they remained active for a varying period of time. Some settled in the minimum time of about 6 days at the laboratory temperature or 12 days at 30° C., while others took about 12 days at the laboratory temperature or about 20 days or more at 30° C. (Tables 2 and 3). A prolonged pre-settling period was correlated with a decrease in size accompanied by well-marked indentations of relatively large areas of the stretched integument of the engorged larva. Engorged larvae which settled in the minimum time showed no appreciable alteration in their appearance. However, long-active larvae ultimately settled quite normally, with the body tilted forwards, abdomen raised, and with the palps and legs adherent to the surface of the filter-paper (Fig. 5B). When all the mites housed in the one cell had settled the cover was usually removed. The settled larvae increased in size but those which had been previously active for a prolonged period, with accompanying reduction of bulk, remained small relative to the size of the others which had passed through a short pre-settling phase.

At the laboratory temperature nymphs emerged between the 31st and 39th day after the introduction of engorged larvae to cells (Table 4). At 25 and 30° C. the time required was not greatly reduced, and it still took an average of 25 days for the nymphs to emerge (Table 5). The nymphs took about 6 hr. from the time when the first active movements were noticed to emerge. When the integument of the settled mite lost its healthy, glassy, tense appearance it was a sign of deterioration, and the somewhat granular condition which ensued was a prelude to collapse, often sudden, of the bulk into a shapeless mass.

Rearing the nymphs to adults

Several authors have recently recommended insect eggs as the best food for the nymphs and adults. Jenkins (1947) used eggs of *Aëdes aegypti* with success, but he pointed out that the young nymphs were unable to pierce the egg-shell and obtain the contents unless the eggs were moistened. Young and old nymphs of *Trombicula autumnalis* when offered moist intact eggs of *Aëdes aegypti* showed no inclination to feed upon them, and they seemed quite incapable of piercing the egg-shell. This almost complete disinterest in the presence of intact mosquito eggs in the chamber of the cell is in contrast to the behaviour of *Trombicula scutellaris* described by Jayewickreme & Niles (1946), who stated that nymphs and adults of this species avidly attacked the offered eggs, firmly held them with the forelegs, pierced the egg-shell, and sucked out the soft parts. Only when the egg-shells were laboriously punctured and the mass of exuded contents was placed around the mouthparts did nymphs of *T. autumnalis* show any signs of feeding. Intact eggs of *Drosophila* stripped of the outer shell were tried, but without the desired success.

Individual nymphs were kept in separate cells and various diets were offered; they included the exuded contents of eggs of various insects (mainly *Aëdes* and *Drosophila*); a mixture of yeast, molasses, and agar, being constituents of a food substrate for rearing *Drosophila*; and chicken faeces (used by Michener, 1946). Nymphs were seldom kept in the same rearing cell during feeding experiments because they will resort to cannibalism if there is a lack

of other suitable food; this feature of their behaviour, common in insects, has been noted by several workers.

A method of 'forced feeding' was adopted to encourage the nymph to introduce the offered food into the gut. Nymphs were placed upon or guided onto the periphery of the soft food mass. Occasionally, a nymph would remain *in situ* and feed continuously until fully engorged, but usually they moved away from the food; when this occurred they were replaced upon it. The process was often repeated many times before a nymph showed any signs of feeding, but sometimes this forced method was of no avail and a nymph would completely resist being forced to take in the offered food. The patience required to make them feed amounted to careful individual nursing. Not one nymph of *Trombicula autumnalis* engorged itself when left unattended in a cell with a quantity of food; if the forced feeding method had not been adopted it is doubtful whether a single nymph would have fed even partially upon the food offered under the experimental conditions.

It was possible to observe under the microscope the flow of the introduced nutrient material. The legs were kept still except for the occasional sluggish movements of the arched forelegs, but the intake of food was accompanied by the alternate up and down movements of the palps with their distal segments vigorously stirring the food material around the mouthparts. Nymphs often moved away from the food in a half-fed condition, whereupon they were replaced upon it; those which fed continuously for about 6–8 hr. became fully engorged. During feeding the body enlarged gradually, the integument flattened out on extension, the identity of the waist region was lost, and the numerous setae became widely separated.

Of the varying diets offered under the conditions of forced feeding, they accepted most easily the mixture of yeast, molasses and agar, but since four of the six adults obtained developed from nymphs which had fed on this mixture plus chicken faeces this appeared to be the best diet. On the other hand, I failed to make nymphs engorge on a diet of chicken faces only, therefore its presence in the diet upon which the nymphs developed may be circumstantial. One came through on a diet of exuded soft parts of insects' eggs (*Aëdes aegypti*) and another developed on a diet of the 'mixture' plus the contents of eggs of *A. aegypti*. Relatively few nymphs became sufficiently engorged with the contents of mosquito eggs to induce further development.

These results are somewhat surprising in view of recent evidence which indicates that nymphs of harvest mites (that is to say those species already tested) grow best on an animal diet, and that the nymphs and adults are predaceous. If by artificial rearing methods the nymphs are enforced or encouraged to engorge on a diet which has the necessary constituents to promote further development, it is immaterial whether or not the nymph is predaceous. The essential requirement is to make the nymph take appropriate nutrient material into the gut, and there is no doubt that the nymph of *Trombicula autumnalis* is able to feed upon nutrient material offered as a soft liquid mass. An important conclusion is that the nymph of *T. autumnalis* showed little or no desire to feed on its own initiative upon the various foods offered, and the feeding behaviour of many nymphs was inexplicable.

Of the individuals selected for further development to the adult stage the mortality rate among them was highest during the first 6 days and a considerable amount of mite material was inevitably sacrificed in the attempts to discover the best conditions for survival. The comparatively few adults obtained emerged after a prolonged pre-adult transition of about 36 days at the laboratory temperature, 100 % R.H. and 28 days at 25° C., 100 % R.H. but these times are based on observations of only six mites. The duration of the pre-adult stage at 30° C., 100 % R.H. was not known because the engorged nymphs kept at this temperature did not survive.

An attempt to force egg laying proved abortive. Large and small adults suspected of being female and male respectively were paired and provided with diets fed to the nymphs. When they showed a detectable increase in size they were exposed to a temperature of 35–37° C. for a period of about 8 hr. and then kept at 25° C. The attempt failed and was disastrous because three adults succumbed and collapsed suddenly into a shapeless mass.

The eggs of harvest mites are spherical, and in the case of *T. autumnalis* it may be assumed that its embryonic pre-larval stage compares with that of *Eutrombicula batatas* and possesses the characteristic dorsal spine and unsegmented appendage sheaths of the intermediate cuticle (dentovum).

THE POST-EMBRYONIC FORMS

Michener (1946) described the main stages of *Eutrombicula batatas*, but in order to make special observations he depended upon the removal of the different stages from his colony, maintained in a medium-sized jar, to a small plaster-of-Paris cell in which the mites did not survive for more than a week or so. He confined his account primarily to taxonomic descriptions rather than to interpreting the peculiarities of progressive development and the nature of the emergence of the active stages.

There was no reference by Michener to any marked variation in the size or general form of either the active or transition stages of *E. batatas*, yet such differences were a marked feature of the post-embryonic forms of *Trombicula autumnalis*. The

transition stages of *Eutrombicula batatas* are, when compared with those of *Trombicula autumnalis*, different in shape and the pre-nymphal stage (protonymph) of *Eutrombicula batatas*, as figured by Michener, is decidedly smaller than the preceding fully engorged larva, a condition never observed for *Trombicula autumnalis*.

Chaetotaxal descriptions have been given for *T. autumnalis* by André (1920) for the nymph and adult, and by Hirst (1915) for the hexapod larva.

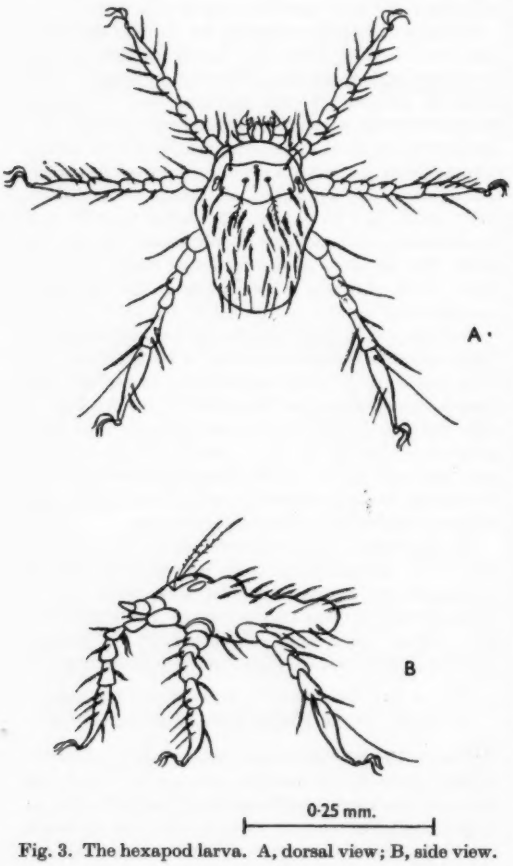

Fig. 3. The hexapod larva. A, dorsal view; B, side view.

The arrangement and number of dorsal setae of the larvae collected around Edinburgh were identical with those of the specimen described by Hirst (1915).

Larva

The free-living hexapod larva is about 0·25 mm. long. Its reddish orange colour is due to the presence of red pigmented spheres associated with the gut. These spheres comprise the pigmented nutrient reserve material upon which the very active larvae depend during their pre-parasitic existence. The eyespots, or ocular areas, are deep red and are arranged one on each side of the posterior lateral border of the dorsal scutum. The legs, each tipped with a trifurcate claw, are pale coloured and very long relative to the size of the body. In dorsal view the body is widest just behind the scutum while the abdomen tapers slightly to the rounded posterior margin (Fig. 3A), but this shape is seldom evident in drawings of mounted specimens. In side view, the anterior half of the larva which supports the legs is more stoutly developed than the dorso-ventrally flattened abdomen, and the two long barbed setae arising from the scutum extend upwards conspicuously above the other setae which are distributed over the body, legs and palps (Fig. 3B).

In unfed larvae some regions of the integument are raised while the intervening furrows, which run over the surface, mark externally the insertions of the muscles underlying the cuticle. The nature of the integument also varies in different parts of the body. Over the inextensible regions, such as the legs and palps, the epicuticle is flat; the endocuticle of the leg and palp segments has well-marked internal ridges for muscular attachment. The chelicerал digits are sclerotized to a pale amber. The epicuticle of the dorsal scutum is distinguished by having its surface pitted with circular depressions of equal size which are arranged closely together but somewhat at random. The margin of the scutum can often be better located by observing the extent of the pitted area rather than by the demarcation ridge when the mounted individual is examined under the microscope. Over the remaining parts of the body the epicuticle of the unfed larva is minutely corrugated to allow for expansion during feeding, while the endocuticle can be extensively stretched. The pattern of the minute corrugations of the epicuticle resembles that of a fingerprint, and the folds are intricately arranged around the circular bases of the sensilla. The epicuticle surface over the base of each seta is smooth, although the periphery of the base itself is raised into a well-marked circular fold. The larger bases of the long barbed setae of the scutum are more intensely folded around the periphery (Fig. 4). The types of setae (sensilla), and their relationship with the sense perceptions of various stimuli of value in acquiring a host, have been discussed in a previous paper (Jones, 1950*d*).

As previously mentioned, the larvae collected around Edinburgh were similar to the specimen figured for Hirst (1915). Richards (1950) examined larvae from different parts of the country and found a well-marked variation of the setal characters, and the extent of this chaetotaxal variation was the basis for proposing eight types in all as variants within the recognized species. Most of the specimens

examined had a simple galeal and dorsal and lateral tibial palpal setae, single scutal setae and single humeral setae, and they were referred to as the normal type. Further division of the normal form was based on the number and arrangement of the post-humeral dorsal setae. Normal type *a* has eight or nine in both the second and third rows; normal type *b* has six in each; normal type *c* has eight in the second row but only six in the third. According to this classification the specimen described by Hirst was a normal type *b* and the individuals collected from localities in Edinburgh and reared in the laboratory also belonged to the normal type *b*, and they compare with the larval type specimen or lectotype. The larvae from various sources in Scotland sent to Dr Richards in 1946 were apparently all normal type *a*, and this circumstantial evidence helped to formulate the view that type *a* was prevalent in Scotland throughout the year, and that type *b* first began to appear farther south in samples from Yorkshire. Type *a* harvest mites were found west of Derby and in Wales, and type *b* and type *c* larvae were found in southern and western England. This distribution of types suggested that distances to the west and north, coupled with regional rainfall and temperatures, were factors favouring the number of setae, and the conclusion reached was that chaetotaxal variation was determined by the nature of the environment during larval development.

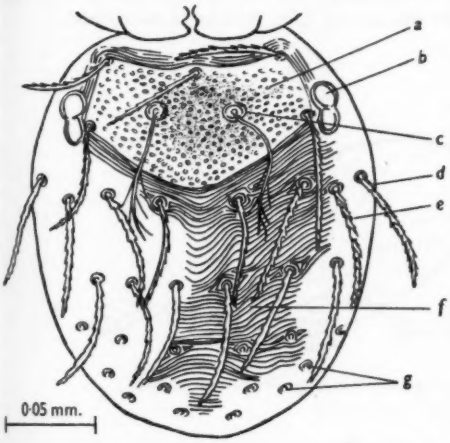

Fig. 4. Detail of cuticle structure and arrangement of dorsal setae. *a*, pitted scutum; *b*, ocular area; *c*, base of seta; *d*, humeral seta; *e*, seta of second dorsal row; *f*, folded epicuticle; *g*, bases of dorsal setae.

The presence of the normal type *b* in Scotland is therefore out of line with the view that regional climate in the British Isles influences ciliation, and that type *a* is consequently confined to Scotland (and the west regions of the British Isles). If one speculated on the presence of type *b* in Scotland, it is possible that type *b* predominated in Scotland in 1946, but, in 1947, which was exceptionally dry and warm, the individuals I recovered in September from loose gravel soil were type *b*. The findings could however be said to fall in line with the suggestion that larvae show the characters of type *b* when they develop in warm, dry (chalky) soils. However, in 1948, July and August were cold wet months in Scotland, but type *b* larvae were recovered from rather heavy moist soil in another district of Edinburgh. Michener (1946), on examining unfed larvae from colonies of *Eutrombicula batatas* cultured at 30° C., 100 % R.H., found that the arrangement of the dorsal setae varied owing to a shift of position of some of the setae so that the dorsal setal formula for the first four post-scutal rows became 2–8–8–9 or 2–8–8–10 instead of 2–8–8–8, and in one specimen 2–9–?–? and in another 2–8–9–?. The indication is that larvae which develop in identical conditions will show chaetotaxal variation, but confirmation could only be obtained by rearing successive generations.

However, the proposal of Richards (1950) that differences in larval ciliation are determined by the environment during larval development is supported by strong evidence, although, as the author admits, it is of a purely circumstantial nature.

When rearing harvest mites in 1947 it was evident that two easily distinguishable types of nymphs were produced; some with long posterior setae arranged in a whorl, and having an exceptionally narrow waist; and others with relatively short posterior setae and a thick waist. In 1949 careful examination of engorged larvae, which gave rise to the two types of nymphs, showed no marked variation in either shape or ciliation, and this led me to change my former belief that perhaps two types of larvae had been cultured, and that they gave rise to two respective types of nymphs. Richards (1950) stated that there appeared a tendency for type *b* larvae to be associated with nymphs possessing long posterior setae, but that type *b* also gave nymphs with short setae; these observations are in line with my laboratory findings.

Engorged larva

To develop further the larva depends upon the opportunity of attaching itself to a vertebrate host to obtain a meal of nutrient material from the malpighian layer of the skin. The larva increases appreciably in size in the first 6 hr. after attachment, and in about 12–15 hr. it seems to have about reached its maximum extension; at 25° C. they remain attached for about 48 hr. at least (Table 1), but the period normally extends from 3 to 5 days.

The fully engorged larva is about 0·55 mm. long, and unless the larva is initially fully fed it will not develop further (Fig. 5A). Some hosts are wandering animals, and there is a tendency for the engorged larvae to be dropped more or less at random over an extensive range of country while hosts like rabbits and field mice keep to set trails so that engorged larvae tend to be dropped in restricted areas. Birds are also common hosts, and I strongly suspect that they are primarily responsible for indiscriminately distributing harvest mites in the gardens of town and city residences which may be far removed from the countryside. It is possible for a garden, perhaps with fruit bushes, to be infected, while surrounding gardens are completely free. I have watched sparrows and starlings hop between rows of raspberry canes and rub their bodies and open wings against soil infected with harvest mites whose remarkable ability for clinging would make the task of climbing on to the bird a comparatively simple one.

The phase in the life cycle when an engorged larva is dropped at random by the host is a vital one because the harvest mite is exposed during most of its life to the microclimatic conditions prevailing in the soil of the locality where it is dropped; the type of microclimatic conditions afforded by the ecological niche then becomes of considerable importance.

In the engorged larva the red spheres, which gave to the body of the unfed larva its characteristic reddish colour, are dispersed in the larger bulk and the body changes to a yellow colour.

In the filter-paper rearing cell the engorged larvae remained active for a time ranging from 6 to 12 days at the laboratory temperature and 12 to 20 days, and occasionally longer, at 30° C., 100 % R.H. (Tables 2 and 3). Engorged larvae which settled in the minimum time retained the bulk size attained at the end of the engorgement period; but those which remained active for the maximum time decreased in size. This condition was accompanied by relatively large regions of the flattened integument becoming indented because the cuticle did not contract uniformly as the contained bulk decreased; this pointed to an absence or elastic recovery of the cuticle. The long-active larvae settled normally with the legs and palps holding the mite in place and the abdomen tilted upward (Fig. 5B).

It was significant that although all the settled larvae gradually increased their bulk by imbibing water, thus further extending the area of the cuticle, those which had been previously active for a comparatively long period remained proportionately smaller, a condition at this stage which appeared to govern the size of the individual throughout post-larval life. Subsequent feeding by the nymphs did not compensate for this initial influence of the pre-settling period upon the size of later stages.

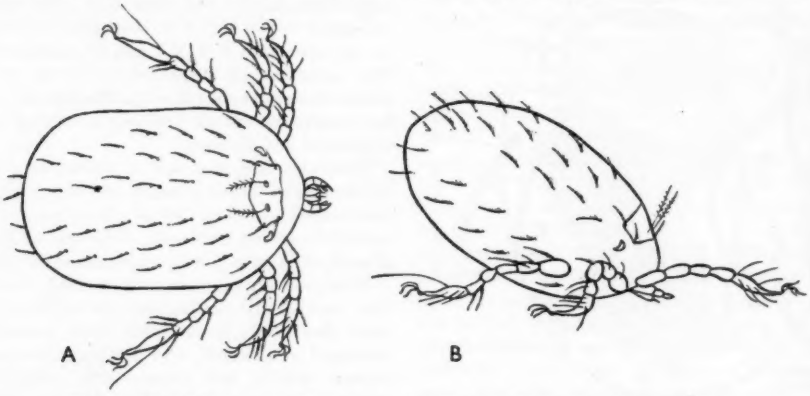

Fig. 5. The fully engorged larva. A, dorsal view; B, stance of settled larva.

The first signs of de-differentiation were evident from the lifeless hollow appearance of the legs and capitulum. Histolysis of the soft parts takes place with subsequent retraction of the disintegrating tissue from the legs and capitulum into the body region, and this re-arrangement of the tissues is accompanied by an accumulation of nitrogenous catabolites in the excretory organ whose outline is defined by the margin of a whitish mass which is clearly contrasted against the pale yellow of the opaque body. The oval form may persist for some time before the tense bulk elongates and assumes the features of the pre-nymphal stage. The larval cuticle is extended to capacity but its presence is recognized by isolated larval setae which project from the surface.

The pre-nymphal stage

Various terms have been used by authors when referring to this particular phase of development. Miyajima & Okumura (1917) called it the nympho-

chrysalis owing to its pupa-like appearance. Ewing (1944) preferred the word protonymph because of its apparent homology with the equivalent stage of other members of the Acarina. However, as Michener (1946) remarked, another member of the Trombiculinae, *Mannquia bequaerti*, has at least four nymphal stages each preceded by a pupa-like transition stage, but in his account he retained the term protonymph. The presence of an intermediate stage. Based on the evidence shown in the development of *M. bequaerti*, mentioned previously, I prefer to regard the features of the transition stage as peculiar to members of the Trombiculinae, for to homologize this stage with the protonymph of the typical life cycle of mites because an extra cuticle is secreted, confuses rather than helps our understanding of this complex development. Michener used the term pre-adult for the stage which precedes

Table 2. *Pre-settling times of engorged larvae of* Trombicula autumnalis at 30° C., 100 % R.H.

Batch	No. of engorged larvae	Date of detachment (1949)	Settling times: Minimum — Date (1949)	Minimum — No. of days	Maximum — Date (1949)	Maximum — No. of days
1	3	6 Sept.	21 Sept.	15	27 Sept.	21
2	3	6 Sept.	21 Sept.	15	28 Sept.	22
3	3	6 Sept.	21 Sept.	15	25 Sept.	19
4	5	6 Sept.	21 Sept.	15	25 Sept.	19
5	6	6 Sept.	20 Sept.	14	28 Sept.	22
6	3	6 Sept.	—	—	24 Sept.	18
7	4	6 Sept.	21 Sept.	15	27 Sept.	21
8	3	6 Sept.	21 Sept.	15	27 Sept.	21
9	11	6 Sept.	18 Sept.	12	27 Sept.	21
10	6	6 Sept.	17 Sept.	11	28 Sept.	22
11	13	13 Sept.	27 Sept.	14	5 Oct.	22
12	12	13 Sept.	24 Sept.	11	3 Oct.	20
13	12	13 Sept.	—	—	25 Sept.	12
14	9	14 Sept.	26 Sept.	12	27 Sept.	13
15	8	14 Sept.	—	—	27 Sept.	13
16	7	13 Sept.	25 Sept.	12	27 Sept.	14
17	8	13 Sept.	24 Sept.	11	27 Sept.	14
18	1	6 Sept.	—	—	7 Oct.	31

Table 3. *Pre-settling times of engorged larvae of* Trombicula autumnalis *at the laboratory temperature*, 100 % R.H.

Batch	No. of engorged larvae	Date of detachment (1949)	Settling time: Minimum — Date (1949)	Minimum — No. of days	Maximum — Date (1949)	Maximum — No. of days
1	9	6 Sept.	12 Sept.	6	17 Sept.	11
2	12	6 Sept.	11 Sept.	5	18 Sept.	12
3	4	6 Sept.	12 Sept.	6	17 Sept.	11
4	10	13 Sept.	27 Sept.	14	—	—
5	9	13 Sept.	17 Sept.	4	25 Sept.	12
6	9	13 Sept.	18 Sept.	5	25 Sept.	12
7	8	14 Sept.	30 Sept.	16	—	—
8	10	14 Sept.	21 Sept.	7	23 Sept.	9

cuticle which, according to the usual tests, has the properties of chitin, and a layer of cells below it have led to the suggestion that the transition stage represents a suppressed protonymph. Cockings (1948) used the term nymphophane.

Sections of these stages clearly showed a relatively thin intermediate cuticle with a distinct layer of large cells with well-defined nuclei below (Fig. 6A). A comparable layer of cells is also present immediately below the intermediate cuticle of the pre-adult the adult, and since it is basically similar to that which precedes the nymph, I see no reason why the name pre-nymph should not be used.

Examination of sectioned material showed that the epidermis secreted the intermediate cuticle, and the layer of relatively large cells beneath this intermediate cuticle is also epidermal in origin, having separated from the epidermis during a later phase of development of the next active mobile stage (Jones, 1950c). The intermediate layer of cells are

homologous with the isolated cells of *Trombidium fuliginosum* (Henking, 1882), and the 'Haemamoebae' of *Atax bonzi* (Claparède, 1868).

Occasionally, as the bulk swells and becomes tense, there is a shift of the cuticular remains of the larval legs and capitulum to one side. The distal segments can be broken off easily and usually these remains collapse into a shapeless mass of disintegrated cuticle which projects from the main bulk phase (Fig. 7B). The larval cuticle persists as the outer covering, but as the bulk elongates and develops further, the ventral swellings which correspond to the legs of the nymph may gradually penetrate the larval cuticle as separate unsegmented tubular sheaths which contain the developing appendages of the nymph (Fig. 7A). The tubular sheaths are part of the intermediate cuticle termed the 'apoderma' by Henking (1882), and the 'Zwischenhaut' by Claparède (1868). This cuticle surrounds the developing nymph in the same way as the deutoval cuticle surrounds the developing larva.

A striking feature of the pre-nymphal stage is the growth of a dorsal spine which is a thickening of the intermediate cuticle. In section the body integument of the developing nymph, below the position of this dorsal spine, is correspondingly raised as a projection (Fig. 6B). According to Nagayo, Miyagawa, Mitamura & Imamura (1917) this dorsal spine is used to pierce the larval cuticle, and Michener (1946) agreed that it has this function, but, although one would expect this to be the case, I do not altogether agree with this view. The dorsal spine and the ventrally placed appendages grow and protrude in opposing directions, thus creating dorso-ventral tension upon the larval cuticle, but it is significant that the tubular leg sheaths which break through on the ventral side appear to be equally if not more responsible for the actual splitting of the larval cuticle. If, as suggested, the dorsal spine first causes a longitudinal split along the mid-dorsal line one would expect the growing tubular leg sheaths to widen the dorsal rent in the larval cuticle. In practically all of the considerable number of individuals examined the reverse is true because the dorsal surface appeared to remain more or less intact, and the wide rents occurred at the ventral surface so that the tubular appendages protruded fully. Occasionally it was difficult to follow the course of the splitting of the larval cuticle, and, prior to emergence, the larval cuticle was sometimes split completely into two halves; the one covering the posterior region; the other, part of the anterior region of the body only (Fig. 8A).

As previously mentioned there was a marked variation in the size of nymphal stages; but each pre-nymphal stage was invariably larger than the engorged larva from which it originated. The smaller forms were about 0·55 mm. and the larger ones about 0·75 mm. The greatest width was across the mid-region; the anterior extremity was mainly composed of the tubular sheaths containing the developing palps and parts of the forelegs of the nymph, while the tapering posterior extremity housed the

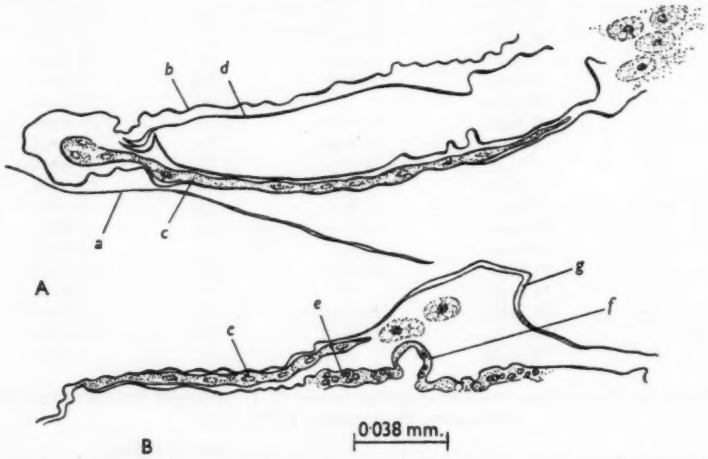

Fig. 6. Portions of sagittal sections of pre-nymphal transition stage. A, portion of tubular sheath with contained nymphal leg and intermediate layer of cells; B, the dorsal spine. *a*, remains of larval cuticle; *b*, intermediate cuticle; *c*, intermediate layer of cells; *d*, cuticle of developing nymph; *e*, nymphal epidermis; *f*, elevation of nymphal integument below dorsal spine; *g*, dorsal spine.

posterior setae. The intermediate cuticle is raised into minute tubercles and it has a regular pattern of depressed hexagonal areas giving a reticulate appearance. The unsegmented tubular sheaths enclosing the developing legs of the nymph are at first arranged close together and they are contiguous with the ventral surface, but eventually they tend to separate and protrude slightly. The cuticle around the body is taut, but the tubular sheaths are much folded or creased and have a loose sac-like appearance. One of the peculiarities of this stage is that its appearance gives the impression that the body and legs are enclosed by a thick cuticle; in fact the covering cuticle is extremely thin and its apparent rigidity is due to the turgescence of the fluid in the exuvial cavity between the definitive integument of the developing organism and the intermediate cuticle (Fig. 6). The larval cuticle tended to remain as an outer continuous covering when the pre-nymphal stages were small but, as described (Fig. 7B), it tended to persist only upon the dorsal surface and extremities of the larger forms.

The white mass of excreta increased in extent with progressive development, and the surrounding yellow-coloured region defined the food-laden ventriculus, while the anterior and posterior regions and the leg sheaths had a translucent grey appearance. For some days before emergence the segmented legs and distal claws of the nymph may be seen through the intermediate cuticle.

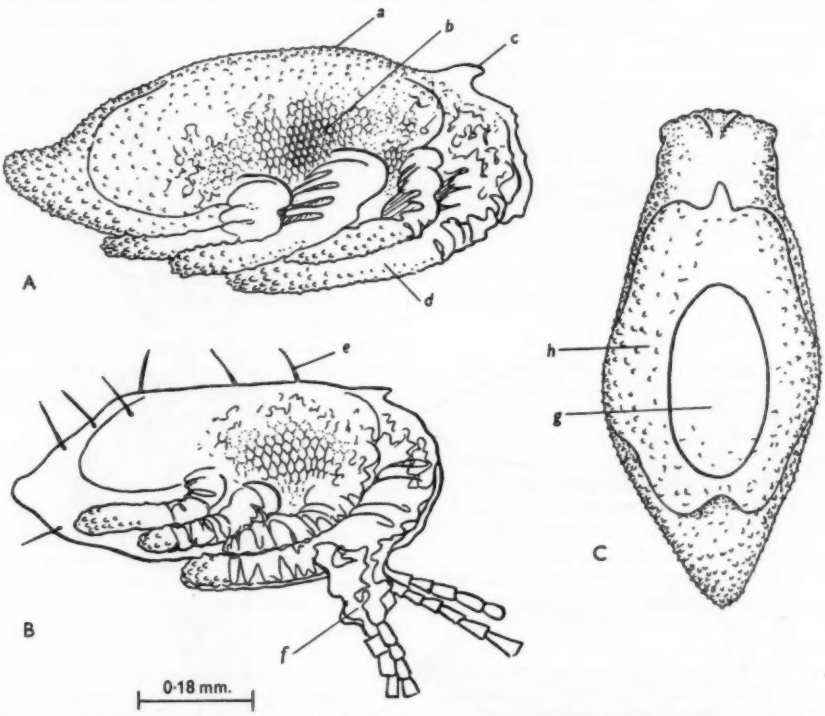

Fig. 7. Pre-nymphal transition stage. A, side view of large pre-nymph; B, small pre-nymph enclosed by larval cuticle; C, dorsal view. *a*, tubercle; *b*, reticulate pattern of the intermediate cuticle; *c*, dorsal spine; *d*, tubular appendage; *e*, larval seta; *f*, remains of larval capitulum and legs; *g*, excrement; *h*, yellow area of food reserves in the ventriculus.

Emergence

The fully formed nymph begins its activity by moving the legs inside the tubular sheaths. Penetration of the larval cuticle presents no problem to the nymph, especially if it has passed through a large pre-nymphal stage, in which case the cuticle has already been mechanically rent with subsequent protrusion of the folded tubular leg-sheaths.

The initial leg movements were a prelude to a rhythmical pulsating of the body. The taut larval cuticle and intermediate cuticle collapse and assume the outline shape of the contained nymph (Fig. 8B, C). The pulsating movements were greatest between the

shoulder regions, but the dorsal spine of the intermediate cuticle, in the centre of this region, cannot possibly have the mechanical function at this stage of splitting the larval cuticle further, even if it persisted, because the collapsed larval cuticle rises and falls in unison with the pulsating body (Fig. 8B, C); cuticle was removed the granular disintegration of the intermediate cuticle was observed more easily, and it was often possible to tease away a colourless viscous substance from the posterior setae. When the cuticular remains of the larval capitulum and legs were placed in contact with the fluid substance

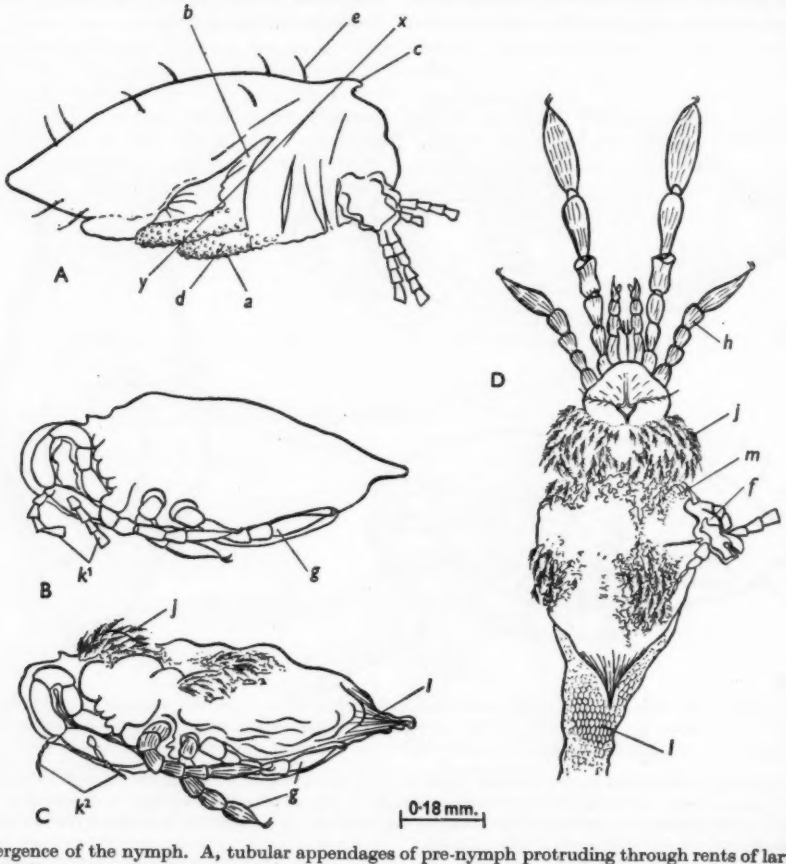

Fig. 8. Emergence of the nymph. A, tubular appendages of pre-nymph protruding through rents of larval cuticle; B, collapse of covering cuticles around pre-nymph; C, nymphal legs and plumose setae breaking free; D, the partially dissolved intermediate cuticle and cast larval cuticle still attached. *a*, tubercle of intermediate cuticle; *b*, rent in larval cuticle; *c*, dorsal spine; *d*, tubular appendages; *e*, larval seta; *f*, remains of larval capitulum and legs; *g*, nymphal leg; *h*, setae stuck to leg surface; *i*, posterior nymphal setae; *j*, nymphal setae; k^1, cuticle remains of larval leg; k^2, cuticle remains of larval leg partially dissolved by moulting fluid; *l*, cast larval cuticle; *m*, margin of intermediate cuticle undergoing dissolution; *XY*, line of tension on larval cuticle.

the peristaltic movements are vigorously initiated in the anterior region and weaken towards the posterior region. Eventually the legs become free from the surrounding sheaths, but the integument of the shoulder region usually emerges first with the characteristic plumose setae gradually becoming poised at an angle to the integument as the covering cuticles disintegrate (Fig. 8C). When the larval on the surface of the emerging nymph, they first softened and then underwent dissolution. The vigorous movements of the body enable the nymph to reject the remains of the larval cuticle, but occasionally a newly emerged nymph will move about with the larval cuticle only partially cast off (Fig. 8D). The disintegrated remains of the intermediate cuticle undergo complete dissolution and

the body setae eventually become dry and erect. The emergence of the adult from the nymphal cuticle and the intermediate cuticle follows the same lines as that described for the nymph.

Michener referred to the nymph emerging through a transverse opening in the anterior part of the dorsum, and the adult emerging through large rents in the dorsal surfaces as if the processes were entirely mechanical, but stated that the intermediate cuticles were not found as exuviae. The observations were only partly valid because the chemical mechanism connected with the emergence was not taken into account. Emergence does in fact depend on the combined effect of vigorous movements and the secretion of a moulting fluid.

Occasionally nymphs would cast the larval cuticle, but they failed to emerge completely from the intermediate cuticle and when such cases occurred it was noticeable that, although the legs were usually free, the body retained the tubular shape of the pre-nymphal form. The surface had a granulated appearance and the setae were pressed against the body, a condition suggesting incomplete dissolution of the intermediate cuticle. When the chemical mechanism failed to operate successfully the nymph seldom survived for more than a day or so.

The nymph

The newly emerged nymph is a pliable, active creature. As the setae become dry and erect the nymph has a whitish yellow fluffy appearance owing to the dense coat of very fine plumose setae which arise from the almost contiguous raised tubercles of the integument. These supple setae become progressively longer towards the posterior. It is the first eight-legged stage, and closely resembles the adult, except that it is smaller and has a more delicate form. The large forelegs are used in the same way as the antennae of insects for testing the immediate surroundings, and at rest the forelegs are poised in the manner of insect antennae (Fig. 9C).

Variation in the size, shape, and setal length of nymphs was a well-marked feature, although the different forms had all developed from outwardly identical unfed larvae. The nymphs obtained may be conveniently divided into small and large forms. The length of the newly emerged small form, measured from the tip of the chelicerae to the posterior margin of the abdomen, was about 0·50 mm.; that of the large one about 0·70 mm.

The nymphs were further divisible into two types; those having typical accentuated shoulders with a well-defined waist region separating them from the larger abdomen, and short posterior setae (α type) (Fig. 9A); and those with a very constricted waist which gives to the body an appearance not unlike that of an hymenopteron insect, but it was the whorled arrangement of the relatively long posterior setae which made this type so easily distinguishable (β type) (Fig. 9B). A comparison of the two types showed no difference in ciliation other than the length and arrangement of the posterior setae. The types were derived from morphologically identical larvae collected in the field at different times during August and September (1949), and all the collections were made in the same locality. Stocks of identical larvae, taken from adjacent pockets of infection within a space of about 9 sq. yd., gave rise to α-type nymphs (with short posterior setae) and β-type nymphs (possessing the long whorled posterior setae).

The excretory organ of newly emerged nymphs is usually charged with nitrogenous catabolites which can be seen through the transparent integument as a distinct whitish mass. Nymphs increase their bulk slightly soon after emergence owing to apparent imbibition of water; the integument extends but the creature retains its pliable appearance; the plumose setae become erect; and the excretory crystals are subsequently rejected.

The pre-adult

The nymphal integument easily maintains its identity because of the presence of the numerous plumose setae. Histo-differentiation and retraction of the material derived from the breakdown of the tissue in the appendages and capitulum of the nymph are expressed by the same kind of symptoms observed in the pre-nymphal stage. Eventually the nymphal appendages and capitulum become brittle and can easily be broken off. The body increases in size and elongates during the period of transformation; it closely resembles the pre-nymphal stage in shape. The nymphal integument was sufficiently transparent to make out the tubular sheaths enclosing the adult appendages and, except for size, the pre-adult was identical in shape with that of the pre-nymphal stage (Fig. 10A–C).

The adult

On emergence the adult appeared to be just as pliable a creature as the newly emerged nymph. It possessed the typical accentuated shoulders and the constricted waist but the general shape was rounder and stouter than the more delicate form of the nymph (see Fig. 11). The adult is also distinguishable from the nymph in possessing three pairs of genital suckers instead of two.

Variation in size, which first became apparent in the pre-nymphal stage, persisted in the adult stage. The larger form was about 1·7 mm. long and the smaller one about 1·5 mm. long, but with respect to shape and ciliation both forms resembled one another. The small- and large-sized adults were associated with small and large pre-nymphal stages respectively; therefore the variation in size of the

adult was linked with a pre-determination phase in development which, as described, appeared to be connected with the pre-settling period of the engorged larva. However, unlike the nymphs, the few adults obtained showed no obvious difference in shape or ciliation. Richards (1950), on examining large numbers of adults collected from the field, found that there were no marked differences in ciliation and that they agreed with the description of an adult given by André (1920). Michener (1946) mentioned that the females of *Eutrombicula batatas* were larger than the males and that this comparative difference in size, correlated with sex, was noted in the pre-adult stage. Examination of the few adult specimens of *Trombicula autumnalis* obtained indicated that the large forms were females and the small ones male, and if the sex is related to size, the determination of the sexes of *T. autumnalis* may be correlated with the pre-nymphal stage or even earlier with the pre-settling period of the engorged larva when the supply of accumulated food reserves seemed to be affected.

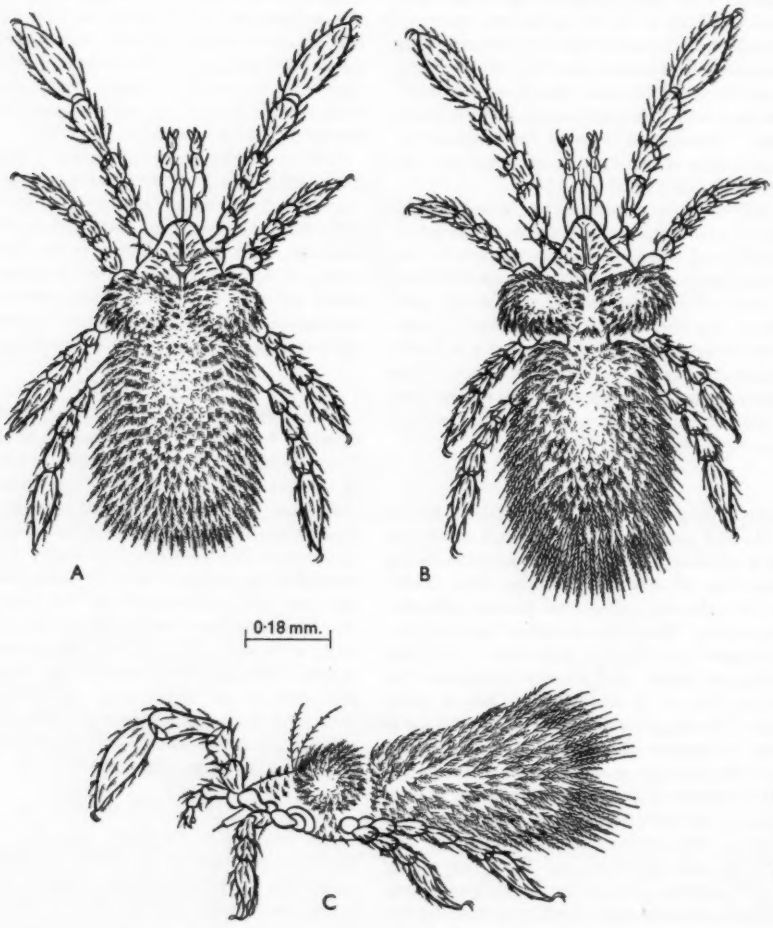

Fig. 9. The nymph. A, α-type; B, β-type; C, side view.

DIMORPHISM

Before considering this special problem it will be convenient to consider briefly the peculiar growth features of the harvest mite and how it differs from that of members of the Crustacea and Insecta in which group dimorphism is not uncommon.

In the harvest mite the pre-nymph and pre-adult are pupa-like developmental phases of the nymph and adult. They are closed systems and in many respects,

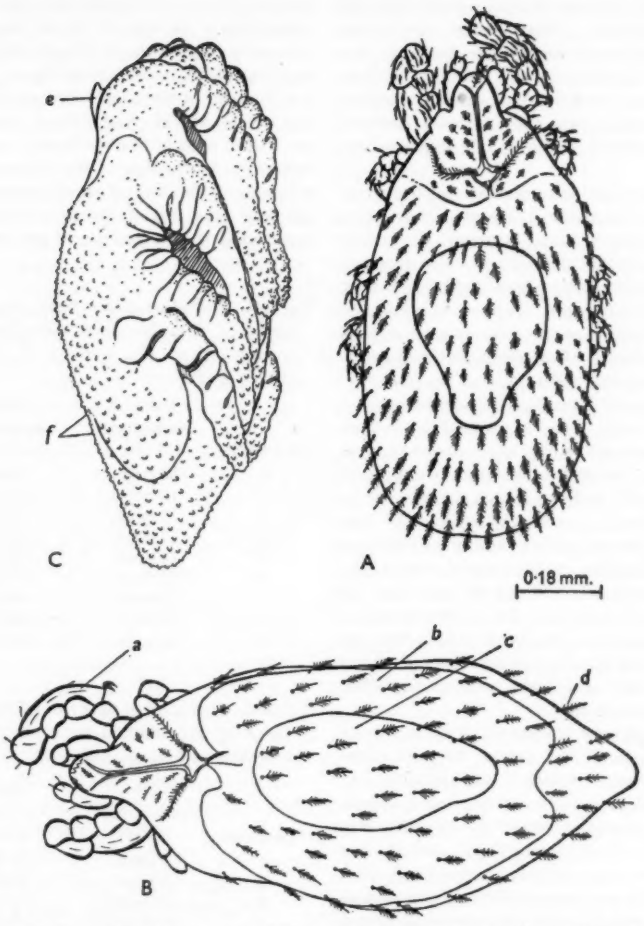

Fig. 10. The pre-adult transition stage. A, fully engorged nymph; B, elongation of form during early development; C, pre-adult dissected from the covering nymphal cuticle. *a*, cuticle remains of nymphal legs; *b*, pale yellow area of nutrient reserves of ventriculus; *c*, white mass of excretory crystals; *d*, plumose seta of nymph; *e*, dorsal spine; *f*, tubercle.

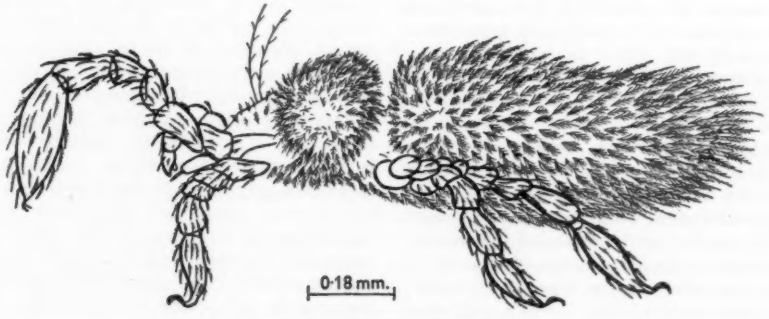

Fig. 11. Side view of adult.

other than this, they compare with the pupal stage of holo-metabolous insects. During the transition period, when the nymphal or adult features, as the case may be, are developing, no further food is taken in, thus growth is dependent on the reserves previously accumulated and the material derived from the breaking down of tissues of the preceding mobile stage.

In the holometabolous insect the developing adult phase, termed the pupa, marks the period of greatest change. On the other hand, during the post-embryonic growth of the harvest mite the greatest developmental changes take place during the first transition stage (pre-nymph) and not during the last one (pre-adult); thus in the harvest mite events with respect to intensity of developmental changes are reversed as compared with those of an insect.

In the harvest mite two kinds of dimorphism exist during post-larval life. One, expressed by a size difference, is connected with sex; the other is confined to the nymphal stage only, when two types of nymphs, distinguishable by obvious differences in body shape and ciliation, were produced. The evidence showed that a state of equilibrium with respect to nymphal dimorphism was attained when the adult stage was reached; the adults showing no variation in shape or ciliation. This observation is in line with the findings of Richards (1950) who, as stated previously, examined great numbers of adults collected from the field and found no marked difference in shape and ciliation.

As previously described, the stocks of unfed larvae collected from the field and reared further, were outwardly identical with respect to size and ciliation. Variation in the time which the engorged larvae took to settle appeared to influence the absolute size of individuals. A short pre-settling period was associated with a consistently large-sized pre-nymphal stage while a long pre-settling period was associated with a small-sized pre-nymphal stage. The period from the time the engorged larva dropped from the host to the time the next nymphal stage emerged, was 31–39 days at the laboratory temperature, 100% R.H., and 20–30 days at 30° C., 100% R.H., and it was significant that small and large nymphs appeared to require these same durations of time to develop fully.

At the laboratory temperature, 100% R.H., the engorged larvae settled after almost continuous wandering in the cell for 6–12 days, and at 30° C., 100% R.H., after 12–20 days. Prolonged locomotory activity was associated with a marked decrease in the bulk size of the engorged larvae, and an accompanying collapse of some areas of the integument which suggested that prolonged locomotory activity was dependent upon a usage of some of the previously accumulated reserves. When this occurred small nymphs were subsequently formed, and when they engorged themselves they never attained a bulk comparable in size to that attained by a fully engorged large nymph which implied that the pre-settling time, when prolonged, not only caused a reduction in the food reserves, but also shortened the actual developing period, hence reducing the growth in area of the epidermis with accompanying reduction in the size of the internal systems. Since subsequent feeding of small nymphs failed to compensate this initial reduction in size, it may be concluded that a prolonged pre-settling time of the engorged larva influences the size of the mite throughout post-larval life. The very limited number of observations made indicated that a size difference was correlated with sex; the small forms being male, and the large ones female.

Table 4. *Duration of time from detachment of engorged larva of* Trombicula autumnalis *from host to the emergence of the nymph at room temperature,* 100% R.H.

Specimen	Date of detachment (1949)	Date of emergence (1949)	No. of days
1	13 Sept.	18 Oct.	36
2	13 Sept.	14 Oct.	32
3	13 Sept.	13 Oct.	31
4	14 Sept.	13 Oct.	31
5	14 Sept.	21 Oct.	39
6	13 Sept.	18 Oct.	36
7	13 Sept.	19 Oct.	37
8	13 Sept.	13 Oct.	31
9	13 Sept.	14 Oct.	32

Table 5. *Duration of time from detachment of engorged larva of* Trombicula autumnalis *from host to the emergence of the nymph at* 30° C., 100% R.H.

Specimen	Date of detachment (1949)	Date of emergence (1949)	No. of days
1	6 Sept.	26 Sept.	20
2	6 Sept.	30 Sept.	24
3	13 Sept.	10 Oct.	28
4	13 Sept.	9 Oct.	27
5	13 Sept.	4 Oct.	22
6	14 Sept.	13 Oct.	30

The setae of the nymphal forms were found to be capable of growing at markedly different rates from the body as a whole. Identical larvae, as described, gave rise to large and small nymphs, but it was clear that they were also capable of developing into one of two types of nymphs (termed α-type and β-type nymphs in this paper) distinguishable from one another by marked differences in the shape of the body, and in the

length and the arrangement of the posterior setae; long posterior setae arranged in a distinct whorl, a hymenopteron-like waist, and accentuated shoulders distinguishing the β-type nymph from the α-type nymph which has short posterior setae, a comparatively thick waist, and consequently less accentuated shoulders.

In a holometabolous insect heterauxetic growth is apparently confined to a relatively brief period, presumably just before and just after the moult from last larval instar to pupa (Huxley, 1932). In the harvest mite heterauxetic growth, which gives rise to two types of nymphs (nymphal dimorphism), is initiated at the beginning of nymphal development (the pre-nymphal stage). This nymphal dimorphism affecting body shape and ciliation gives way to a state of equilibrium during the developing stage of the adult (pre-adult stage). Therefore, it appears that nymphal dimorphism in the harvest mite is of developmental origin and that the α-type and β-type nymphs are not of genetic origin. Experimental rearing of mites from outwardly identical stocks of larvae under regulated conditions also suggested that nymphal dimorphism in the harvest mite is independent of the external environment.

The features which distinguish the α-type and β-type nymphs are associated with the epidermis. One must therefore suppose that some latent mechanism, or morphogenetic chemical system, residing within the epidermal cells can acquire different properties of growth according to the influence of some growth factor or factors. That the epidermis of the harvest mite is capable of acquiring different properties at some given phase during progressive growth is expressed by the production of α-type and β-type nymphs from identical larvae.

SPECULATION ON THE LIFE CYCLE IN THE NATURAL ENVIRONMENT

The results of experimental rearing of *T. autumnalis* in the laboratory permits speculation on the life cycle in the natural environment. Cockings (1948), and Richards (1950), established the presence of adults in the soil throughout the year. In tropical countries the nymphs and adults are found, at least during the wet season, on the surface of the soil; they are easily obtained by searching in the leaf mould and debris, or under stones or logs upon infected ground, but some species, for example, *Eutrombicula batatas* and *Trombicula scutellaris*, are abundant upon the actual surface of the soil. According to Michener (1946) the life cycle of *Eutrombicula batatas* can be completed in about 25–45 days at 30° C. when reared in the laboratory, which suggests that more than one generation of larvae of a tropical species could be produced in a year.

At 30° C., 100 % R.H., it takes about 60 days to rear *Trombicula autumnalis* from the engorged larval stage to the adult; at the laboratory temperature it takes at least about 70 days (Table 6). The development of *T. autumnalis* was accelerated only at the expense of heavy casualties by exposure to 30° C.; individuals thrived best under conditions approximating to those met with in the natural environment.

According to the tabulated times which the various stages of *Eutrombicula batatas* require (Michener, 1946) the incubation period of the egg, and the period of the pre-larval stage of a harvest mite, are each equivalent to the duration of either the pre-nymphal or the pre-adult stage. Therefore the time required for *Trombicula autumnalis* to develop and grow from the egg to the adult under the artificial conditions of experimental rearing when food is made available soon after the growing stage has emerged, would be about 144 days or 4–5 months (Table 6). In the natural environment an unfed larva may not acquire a host for 3–4 weeks; similarly, a nymph may not satisfy its food requirements for some considerable time. Therefore both these fluctuating periods of time governed by the availability of food in the particular niche where the mites happen to be living, may well extend the life cycle by 1–2 months. It is known that the numbers of unfed larvae reach a peak in September, so it is probable that a good proportion of the seasonal population will enter post-larval life at the approach of winter, but, even if a slowing up of the life cycle owing to low temperatures is ignored, the minimum duration would be about 6 months from oviposition to the emergence of the adult.

Engorged larvae which drop off the host and return to the soil in July, according to the above estimate, should attain the adult stage about December; those of August in January; and those of September in the spring. The effect of food and temperature upon the maturity of the adults, and temperature and humidity on the incubation period of the eggs, would govern the early or late appearance of broods of unfed larvae upon the surface of the soil. The main season in Scotland is from July to the beginning of October (Jones 1950*a*), and farther south from July to November (Richards, 1950), with September the peak month in both cases. Evidence therefore points to there being one generation of larvae of *T. autumnalis* each year; but successive broods are produced during the season.

The pre-settling time of the engorged larvae of tropical harvest mites is considerably less than that of our own harvest mite. At 30° C., 100 % R.H., engorged larvae of *Eutrombicula batatas* settle in 2 days (Michener, 1946) while those of *Trombicula autumnalis* take 12–20 days. The difference between the two species with respect to this pre-settling time would appear to be inherent and correlated with the

Table 6. *The post-embryonic growth of* Trombicula autumnalis *in the laboratory*

Growing stage	Transition stage	Features	Phase	Average duration at the laboratory temperature, 100 % R.H.	Average duration at 30° C., 100 % R.H.
Larva	—	Locomotory with six legs; reddish orange colour; bright red ocular areas on each side of scutum; transverse rows of tactile setae on dorsal and ventral surfaces of body; tactile setae and peg organs on legs and palps	Free-living	21 days in moist sterile soil	—
			Feeding	3–5 days	2–4 days
			Pre-settling	6–12 days	12–20 days
	Pre-nymph	Immobile and pupa-like; larval cuticle forms outer covering; dorsal spine, tubular sheaths and tubercles of intermediate cuticle; nymph entirely enclosed by intermediate cuticle; size varies; intermediate cells of epidermal origin in the exuvial cavity	Change	24 days	11 days
Nymph	—	Locomotory with eight legs; whitish yellow colour; forelegs used like insect antennae; body and legs heavily clothed with plumose setae; size varies; two types of large nymphs which vary in shape and ciliation; two pairs of genital suckers	Emergence	6–8 hr.	
			Free-living	23 days in moist sterile soil	—
			Feeding	6–8 hr. to become fully engorged if feeding is continuous	—
			Pre-settling	Usually remains at site after continuous feeding	—
	Pre-adult	Nymphal cuticle forms outer covering; in other respects, except for size, it resembles the pre-nymph	Change	36 days	— 28 days (25° C.)
Adult	—	Locomotory with eight legs; yellowish white colour; larger and more robust than the nymph; body and legs heavily clothed with plumose setae; indication of small forms being male and large ones female; no apparent variation in ciliation; three pairs of genital suckers	Free-living	Observed for 7 days only, but evidence suggests that in the natural environment its active life is very prolonged and extends at least from the winter to the summer	—

conditions of their respective natural environments. The engorged larvae of tropical harvest mites, when they drop from a host, only require to find shelter under leaves, stones, debris, or an inch below the surface of the soil because deep penetration into the soil is unnecessary in a climate which provides appropriate warmth and moisture for further development of the post-larval stages at the surface of the ground. Hence, the tropical harvest mite has little difficulty in finding a suitable niche before the onset of the transition stage. On the other hand, engorged larvae of the British harvest mite, even when dropped by chance into a suitable niche, must penetrate the soil for a considerable depth so that the subsequent transition stage will be protected from adverse drought and low autumn and winter temperatures. To judge from the rate of movement of an engorged larva it is capable of lateral movement upon the surface of the ground, and it is probably capable of seeking a more appropriate habitat if the site where it is dropped by the host is unsuitable for burrowing downwards. The long pre-settling period of an engorged larva of *T. autumnalis* is inherently regulated, and is presumably an adaptation which allows the engorged larva time to penetrate the soil to a suitable depth to acquire an appropriate niche which will enable the harvest mite to be independent of the seasons of the British Isles.

SUMMARY

1. *Rearing.* Larvae of *Trombicula autumnalis* were fed on young mice (about 8 days old); after 48 hr. at 25° C. they began dropping off fully engorged.

The post-larval stages were reared individually in separate filter-paper cells. At room temperature, 100% R.H., the engorged larvae were actively mobile for about 6–12 days, and for 12–20 days at 30° C., 100% R.H., before entering a quiescent stage (the developing nymphal stage). The nymphs emerged after about 33 days at room temperature, and 25 days at 30° C., from the time the engorged larvae dropped off the host.

Of the nymphs which developed to adults, four fed on a mixture of yeast, molasses, and agar plus chicken faeces; one fed upon the exuded contents of the eggs of *Aëdes aegypti*; and another fed upon a mixture of all the ingredients mentioned. Young and old nymphs of *Trombicula autumnalis* appeared to be incapable of feeding upon intact insects' eggs; neither did they show any particular predilection for the exuded contents of insects' eggs offered as a soft mass, and only with some difficulty were they made to introduce this food into the gut. When chicken faeces were offered as food the nymphs did not feed upon it.

A method of 'forced feeding' was adopted, that is to say, nymphs were placed upon or guided on to the periphery of the food mass provided. When a nymph moved away it was replaced upon the food, and the process was repeated until the nymph showed signs of introducing the food into the gut. Occasionally a nymph after being placed upon the food remained *in situ* and fed continuously for about 6–8 hr. until completely engorged.

2. *The post-embryonic forms.* The features of the stages (the larva nymph and adult) are described with respect to their development and growth.

The transition, or developing stages (the pre-nymph and pre-adult), are closed systems which resemble the pupal stage of a holometabolous insect. The developing mite is enclosed by the cuticular remains of the preceding mobile stage, and a second inner or intermediate cuticle. Large flattened cells of epidermal origin are present, usually lying against the inner surface of the intermediate cuticle. Emergence of the nymph and adult depends upon the combined effects of an active secretion, which causes a disintegration of the intermediate cuticle, and muscular exertion.

3. *Dimorphism.* Two kinds of dimorphism are shown during the growth of *T. autumnalis*; sexual dimorphism associated with size, and nymphal dimorphism depending on marked differences in body shape and in the length of the posterior setae.

Both kinds of dimorphism are initiated at the beginning of post-larval life (beginning of nymphal development).

The time which the engorged larva takes to settle is inversely proportional to the size of the post-larval stages; in other words, the amount of activity and accompanying usage of acquired food reserves before the engorged larva settles appear to govern the absolute size of the individual during post-larval life. There is an indication of small forms becoming male and larger ones female.

Two types of nymph (referred to in this paper as α-type and β-type nymphs) were bred from outwardly identical larvae. The differences in the growth of the body as a whole and the posterior setae, which distinguish the two types of nymph, appear to be of developmental origin.

4. *The life cycle in the natural environment.* Laboratory findings confirm the view that only one generation is produced each year, but that successive broods are produced throughout the season of incidence.

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EGYPTIAN FEVER OF CATTLE

THE TRANSMISSION OF *THEILERIA ANNULATA* (DZCHUNKOWSKY & LUHZ 1904) BY *HYALOMMA EXCAVATUM*, KOCH, 1844

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(With 7 Figures in the Text)

GENERAL

From September 1946 onwards, for a considerable period, the senior author was closely occupied at the Serum Institute, Abbassiah, with the production and titration of goat-adapted cattle-plague virus, and with various experiments on cattle plague. Cattle imported from Cyrenaica and Cyprus, and local Egyptian cattle, were used as experimental animals in roughly equal proportions; and these cattle were kept, sometimes for several months, in open lines on the premises of the Veterinary Infirmary, some 400 yards from the Serum Institute, before being drafted for experimental work. The Cyrenaican and the Egyptian cattle were usually 9–18 months old, while the Cyprus cattle were often fully grown. It was observed that when such animals were placed in an isolation unit at the Institute and were treated with cattle-plague virus in the form of goat tissues or chick-embryo preparations, a great many of them developed an attack of theileriasis (Egyptian fever), which might begin any time between 11 and 30 days after the experimental observation had started, but more often between the 11th and the 20th days.

Apparently, it had for a long time been the generally accepted view in Egypt that similar attacks of Egyptian fever in newly purchased Cyprus cattle introduced to the premises of the Serum Institute were either relapses in premunized animals, precipitated by the reactions that followed an injection of cattle-plague virus, or breakdowns of immunity resulting from the injection of blood or tissues containing viable schizonts of a *Theileria*. However, in this series of observations it was soon realized that the attacks of theileriasis developed, irrespective of whether or not there was any reaction to the inoculated materials, and in no case was the material of such a nature that viable schizonts of *Theileria* could have been injected.

From the autumn of 1946 to the early summer of 1947 the only ticks noticed on the animals were a few adults of a *Hyalomma* species, feeding on the scrotum and perineum or in the inguinal and axillary regions, and a few specimens of *Boophilus* or *Margaropus* on the body; and during the months January–April the *Hyalomma* adults were rarely seen. Later, during the summer of 1947, nymphae of *Hyalomma* were taken in large numbers from an occasional animal, to which they were attached chiefly along the crest of the neck.

In view of what Sergent, Donatien & Lestoguard (1945) have called the 'rupicolous' habit of *Hyalmoma mauretanicum*, the vector of *Theileria dispar*, searches were made in the buildings of the isolation unit, but it was not until the autumn of 1947 that wintering ticks—mainly unfed adults, but also a fair number of engorged nymphae—were discovered in large numbers in a patch on the outside of the brick wall of the building, facing west, and extending from a foot above ground-level to a height of 6 or 7 ft. The ticks were fairly well concealed in cracks and crevices between the brick courses, which were finished with a 'wipe' joint. The mortar, that is to say, had been wiped away with a bluntly pointed instrument, so that the edges of the bricks projected from ¾ to 1½ in. beyond the level of the mortar. Where the mortar was fissured, or where an accumulation of dust or spiders' webs had provided cover, the ticks lay, squeezed into horizontal or vertical crevices or merely flattened against the surface of the mortar, with their legs contracted. They were perfectly quiescent and apparently quite resistant to the effect of desiccation during the dry, sunny Egyptian winter. The resistance to desiccation of the unfed adults and engorged nymphae of this species is quite remarkable. Where suitable cover is not available, adult ticks have been seen surviving the winter flattened against a concrete-plastered wall, taking advantage of such slight cover as that which is afforded by proximity to a dried-out splash of cowdung.

When, during the winter, the adults are taken from their hibernating site, they immediately become fully active in the hand or in a test-tube; but it is noticeable that cases of the disease are less frequent during the winter months of January, February and March, and that, if cattle are tethered during those months close to the sites of hibernation, very few ticks leave these sites to attach to them.

Hibernating adults have been fed successfully in each of the winter months, but the ticks do not feed as well as they do in the summer. Whenever hibernating ticks have been fed on susceptible cattle, they have transmitted infection; but unfortunately there has not yet been an opportunity to feed hibernating adults on susceptible cattle during February and March.

MONTHLY FREQUENCY OF CASES AND COMPARATIVE SUSCEPTIBILITY OF CYRENAICAN, CYPRUS AND EGYPTIAN CATTLE

The monthly frequency of theileriasis in the three types of cattle introduced into the infested buildings is tabulated in Table 1. The criterion of infection is the occurrence of a febrile attack, usually lasting several days, which may or may not be accompanied by other symptoms such as swelling of the superficial lymphatic glands, refusal to feed, deep injection of the visible mucous membranes, occasional diarrhoea, and general dullness. Schizonts of *Theileria*, chiefly agamonts, are found in the early stages of the attack in the enlarged lymphatic glands, and later both types of schizont (gamonts and agamonts) may be seen in blood films, together with gametocytes, which may infect from 5 to 80% of the red cells, depending upon the severity of the attack. Should the animal die, the post-mortem lesions are those generally characteristic of theileriasis, wherever it has been described.

The table affords a rough indication of the comparative susceptibility of the different breeds, as well as an index of the seasonal activity of the vector. It will be noted that no Cyprus cattle were exposed during the last 4 months of the year, but the figures for the other breeds do not suggest that this fact has lowered the recorded frequency of cases in this breed, since three of the months, September, October and November, are the three which show the highest quarterly incidence of cases in the other two breeds.

The peak months for transmission are quite clearly August to November, and the lowest December, January and February. The peak is reached when both nymphae and adults are operating as vectors, the lowest point when engorged nymphae and unfed adults are hibernating in the buildings. It will be noted, however, that natural infections can, and do, occur in every month of the year. This is consistent with the experience of Delpy (1949*a–d*) with theileriasis in Iran transmitted by a three-host tick, and in contrast to the experience of Sergent and his fellow workers with *T. dispar* transmitted by a two-host tick, in Algeria, where there is definitely a close period of several months in which cases do not occur (Sergent *et al.* 1945).

TRANSMISSION BY NATURALLY INFECTED TICKS

The first experimental transmission was effected with adult ticks reared from nymphae that were taken from the crest of the necks of two Benghazi calves, nos. 88 and 89, that were suffering, in June 1947, from naturally contracted attacks of theileriasis in the cattle-plague isolation units. Ten females and eleven males were placed on Benghazi calf no. 87, on 29 August 1947. The calf was kept at the Infirmary, where there had been no evidence of the presence of *Hyalomma*. Two of the females engorged

Table 1. *Monthly incidence of natural infections at Abbassiah*

Month	Total, all breeds: Percentage infected	Total, all breeds: No. infected / No. exposed	Egyptian cattle: No. infected / No. exposed	Cyrenaican cattle: No. infected / No. exposed	Cyprus cattle: No. infected / No. exposed
September	77	17/22	7/12 (1+)*	10/10 (2+)*	—
October	75	9/12	—	9/12 (2+)	—
November	60	12/20	—	12/20 (6+)	—
December	16·6	4/24	0/2	4/22 (1+)	—
January	11·5	4/26	0/15	4/9 (1+)	0/2
February	11·5	3/26	1/20	0/1	2/5
March	33·3	10/30	2/16	4/7	4/7
April	36	8/22	4/11	3/10	1/1
May	25·5	3/12	—	1/10 (1+)	2/2
June	32	7/22	—	4/19 (2+)	3/3 (1+)
July	39	8/18	2/5	2/9	4/4
August	100	3/3	3/3	—	—
Total	33	88/237	19/84 or 22·6% 1 Death	53/129 or 42% 15 Deaths	16/24 or 66/6% 1 Death

* 1+ signifies 1 death.

promptly, and dropped on the 8th and 9th days. A febrile reaction developed on the 8th day, and calf no. 87 exhibited a characteristic, fairly mild, attack of theileriasis, lasting 7 days and ending in recovery. Schizonts were demonstrable in gland smears from the 9th to the 15th day, and gametocytes in blood films, from the 14th day (see Fig. 1).

dropped fully engorged between the 8th and the 21st day. The calf reacted on the 9th day, suffering a typical attack of theileriasis with parasites in the blood and gland (see Fig. 2).

(2) *Adult* ticks collected from the walls of the isolation building on 27 November were fed on susceptible Benghazi calf no. X 86 on the same day.

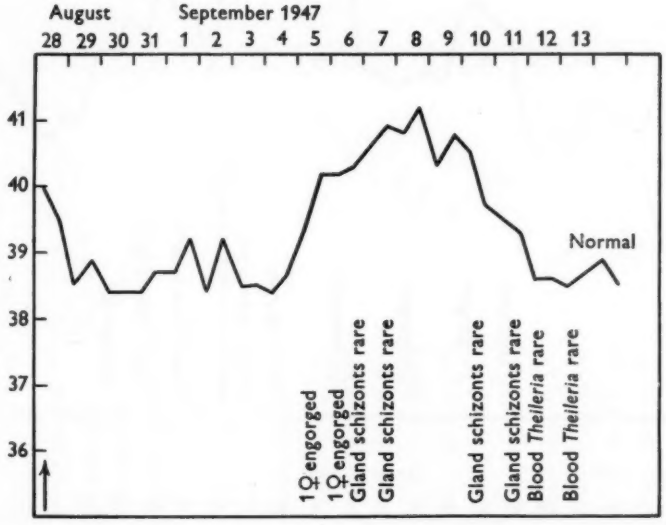


Fig. 1. Calf no. 87, infected by adult ticks reared from naturally infected nymphs.

Another collection of wild nymphae was made from Benghazi calf no. X 15, which died from naturally contracted theileriasis on 3 November 1947 in the isolation unit. Adults reared from these nymphae were fed on 30 March and 18 May 1948 on Cyprus bull no. 152 and Benghazi calf no. 199 respectively. The females on Cyprus bull no. 152 fed poorly, the last engorged specimen dropping on the 17th day. A mild febrile reaction lasting 2 days developed on the 19th day. Parasites could not be found in blood or gland smears, but no. 152 was subsequently found to be immune when it was challenged by the feeding of hibernating ticks which transmitted the disease to a susceptible calf. Benghazi calf no. 199 reacted on the 13th day and exhibited a mild attack of theileriasis with fever and a few gametocytes, and an infection of erythrocytes which never exceeded 5%.

HIBERNATING TICKS

(1) *Adult* ticks, 14 ♀♀, 3 ♂♂, taken from the interstices of the walls of the isolation building on 25 November 1947, were fed on the scrotum of susceptible Benghazi calf no. X 88. Eight of the females

Calf no. X 86 reacted on the 10th day and suffered a severe clinical attack of theileriasis with numerous parasites in the blood and glands.

(3) *Adults*, 15 ♀♀ and 5 ♂♂, taken from the isolation building were fed on 1 January 1948 on susceptible Benghazi calf no. X 76. Engorged females dropped between the 9th and 17th days, and the calf reacted severely on the 9th day with a clinical attack of the disease, showing numerous parasites in blood and gland smears. Infection of red cells reached a maximum of 80%.

(4) *Larvae* derived from a single engorged female of the hibernating ticks that infected Benghazi calf no. X 86, *vide supra*, were fed on susceptible Benghazi calf no. 185 in May 1948 without evoking any reaction. The derived nymphae were fed on Cyprus calf no. 157 on 13 July 1948, which at the time was exhibiting a severe naturally contracted attack with fever and numerous *Theileria* in the blood, reaching a maximum of 70% red cells parasitized, when the nymphae began to feed.

(5) *Adults* derived from these nymphae were fed on susceptible Benghazi calf no. 205, on 9 August 1948. Calf no. 205 reacted on the 7th day, and suffered a

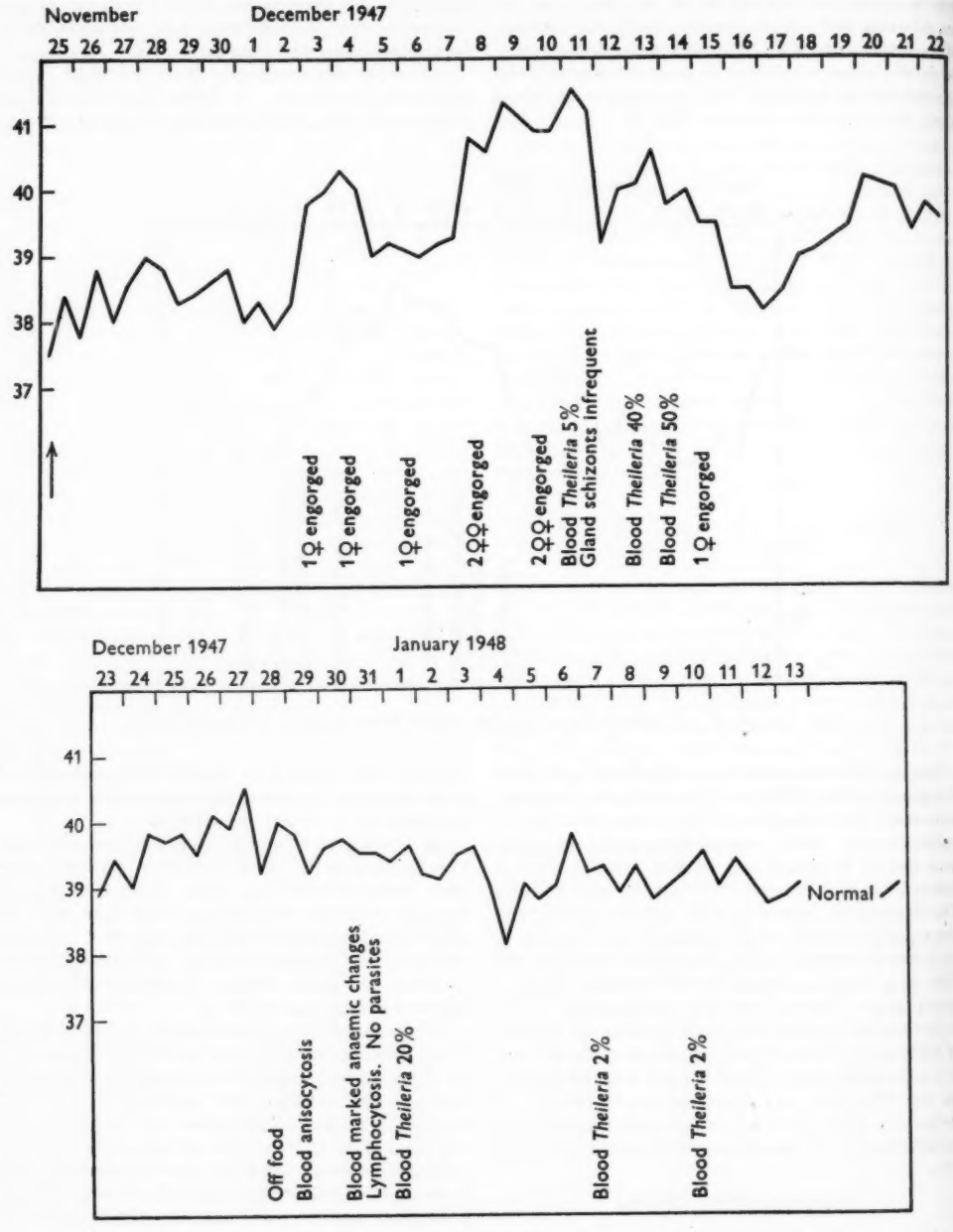


Fig. 2. Calf no. X 88, infected by adult ticks hibernating on the walls of an isolation building.

prolonged and severe attack of theileriasis, with parasites in the blood reaching a maximum of 40 % red cells infected (see Fig. 3). On 19 September 1948 a further batch of these adults (7 ♀♀ and 4 ♂♂) were fed on susceptible Benghazi calf no. 166. This calf also reacted on the 7th day and developed a typical, but relatively mild, attack of theileriasis, with fever and parasites in the blood.

Adults from these nymphae were fed on 30 October 1947 on susceptible Benghazi calf no. X 88, the calf that had been used to feed the nymphae. Again there was no reaction to the feeding.

Second generation

Larvae of the second generation were fed on Benghazi calf no. 199 which had recovered from a

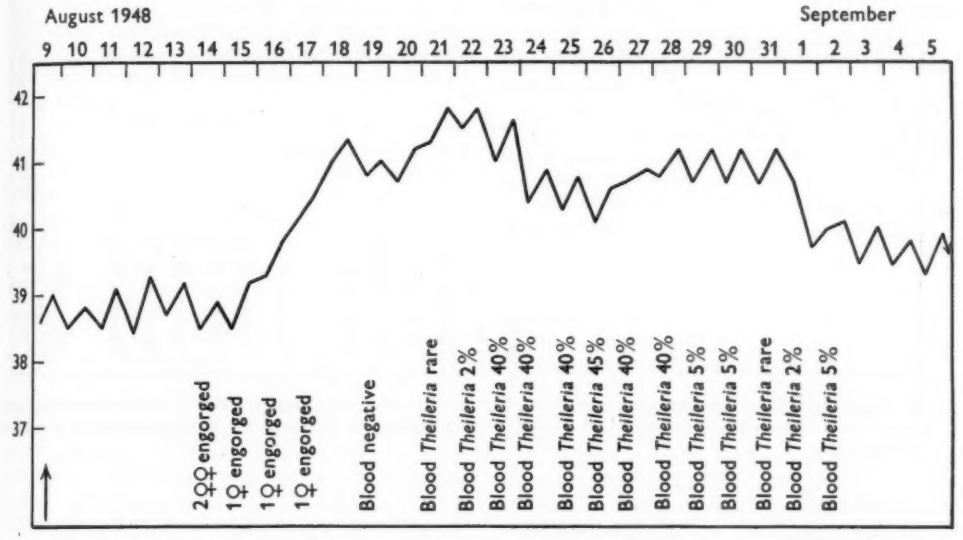


Fig. 3. Calf no. 205, infected by adult ticks derived from nymphs fed on a naturally infected calf. These nymphs came from a hibernating tick.

TRANSMISSION WITH LABORATORY-BRED TICKS

In view of the great variation in size and macroscopic characters generally shown by adults reared from 'wild' nymphae, and later by the hibernating adults, and of the extreme difficulty at that time of determining species of the genus *Hyalomma*, it was decided to attempt transmission of *Theileria* with two lines bred from a single female. Accordingly, a single engorged female was taken on 25 June 1947 from Benghazi calf no. 122 which at the time was suffering from a naturally contracted acute attack of theileriasis.

First line

First generation

Larvae. Larvae raised from the eggs laid by this female were fed on 28 August 1947 on susceptible Benghazi calf no. 90 which did not react to the feeding.

Nymphae moulted from these larvae were fed on 26 September 1947 on susceptible Benghazi calf no. X 88 which did not react.

fairly mild attack of theileriasis (1–6 June 1948), induced by the feeding of adult ticks of lot 5. At the time the larvae were fed (3 July 1948) parasites were extremely rare in the red cells of calf no. 199.

Nymphae. Twenty-two nymphae derived from these larvae were fed on 23 July 1948 on susceptible Benghazi calf no. 188 which had already been used for feeding some of the larvae of the second generation, *vide infra.* Calf no. 188 reacted on the 17th day to the feeding of these nymphae, showing transient fever (2 days) and a slight blood infection with *Theileria.*

Larvae. Another part of this batch of second-generation larvae was fed on susceptible Benghazi calf no. 188 on 16 June 1948, i.e. 37 days before the infective feeding of nymphae recounted in the previous paragraph. Calf no. 188 did not react to the feeding of larvae.

Nymphae. Nymphae, derived from larvae fed on calf no. 188 when susceptible, were fed on 8 July 1948 on Cyprus bull no. 159 towards the end of a naturally contracted attack of theileriasis, during which schizonts were seen in gland smears, although

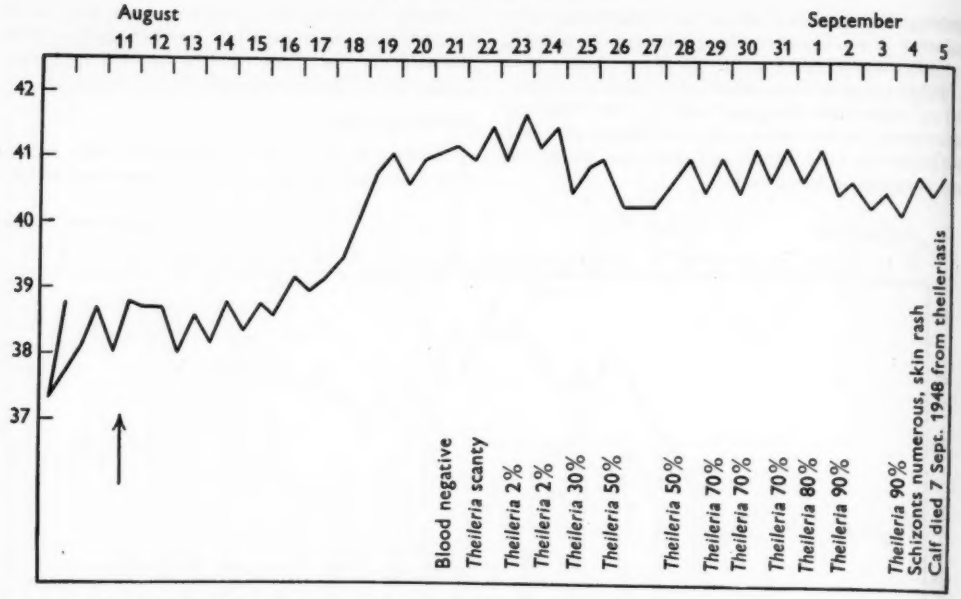


Fig. 4. Calf no. 185, fatal infection conveyed by adult ticks of the second generation reared in the laboratory from nymphs fed on a naturally infected bull. These nymphs came from larvae of the second generation fed on an infected calf.

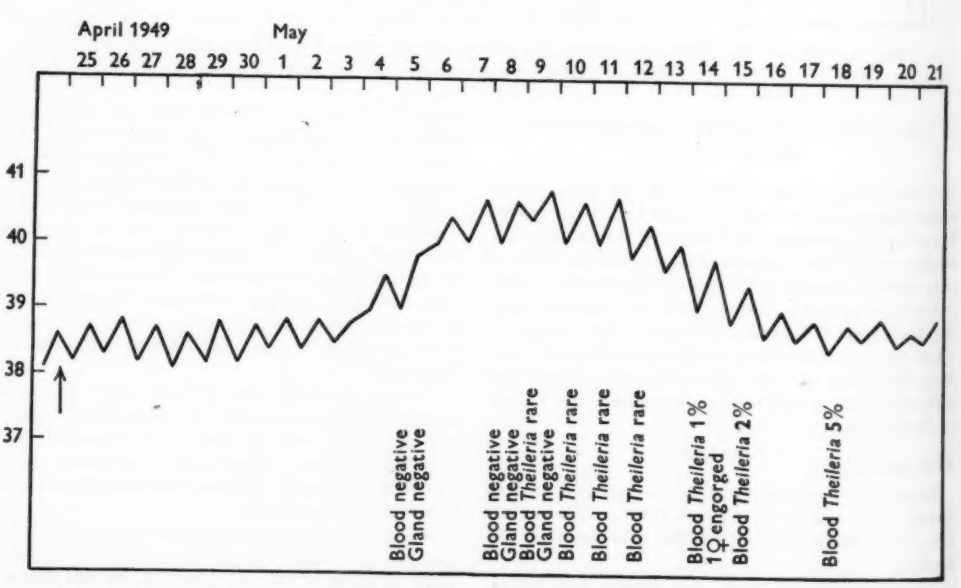


Fig. 5. Calf no. 282, infected by feeding adult ticks of the third generation reared in the laboratory from nymphs that had fed on the experimentally infected calf no. X 88, whose infection is shown in Fig. 2.

gametocytes could not be found in the blood cells.

Adults. Adults derived from these nymphae were fed on 11 August 1948 on susceptible Benghazi calf no. 185 which reacted on the 8th day, suffering a severe attack of Egyptian fever which lasted 19 days and ended with the death of the animal (see Fig. 4).

Third generation

Larvae. Larvae of the third generation derived from three engorged females that dropped from Benghazi calf no. 185 were fed on susceptible calf no. 286 on 11 January 1949. This calf did not react to the feeding.

Nymphae. Nymphae derived from these larvae were fed on 1 and 4 February 1949 on Benghazi calf no. X 88 which had recovered from theileriasis induced by the feeding of hibernating ticks on 25 November 1947. Clinical recovery was apparently complete by mid-January 1948, but this calf exhibited a mild relapse lasting 2 days in February 1948 following a reaction to goat virus of cattle plague.

Adults. Adults moulted from the nymphae that had fed on the recovered Benghazi calf no. X 88 were fed on susceptible Benghazi calves nos. 255 and 282 on 3 and 24 April 1949 respectively: four males and six females on no. 255, and three males and five females on no. 282. Calf no. 255 contracted theileriasis, the reaction beginning on the 10th day. Calf no. 282 also contracted theileriasis as a result of this feeding, the attack beginning on the 10th day and lasting 12 days (see Fig. 5).

Nymphae. Another batch of the nymphae that had been fed as larvae on susceptible Benghazi calf no. 286 was fed on Benghazi calf no. 235 on February 1949. Calf no. 235 had recovered from an attack of theileriasis earlier in the same month.

Adults. Adults derived from nymphae fed on recovered calf no. 235 were fed on susceptible Benghazi calves nos. 286 and 267 on the 14 and 28 April 1949 respectively. Both calves reacted on the 10th day, exhibiting clear clinical attacks of Egyptian fever with parasites in the blood and glands, ending in recovery. Some of the same batch of adults were fed on calf no. 235, the infecting host of the nymphae, with the object of testing its immunity. Calf no. 235 did not react.

Nymphae. A third lot of nymphae from the same batch was fed on susceptible Benghazi calf no. 287 which did not react to the feeding.

The batch of adults derived from the nymphae fed on susceptible calf no. 287 were not fed.

Second line

First generation

Further lots of larvae derived from the single engorged female from reacting calf no. 122 were fed on susceptible Benghazi calf no. X 86 on 23 September 1947. The calf did not react to the feeding.

Nymphae derived from these larvae were fed on susceptible Benghazi calf no. X 88 on 23 October 1947. The calf did not react to the feeding.

Adults from these nymphae were fed on recovered Benghazi calf no. 76 on 20 April 1948. There was no reaction.

Second generation

Larvae of the second generation were fed on 13 July 1948 on Cyprus calf no. 157 during an attack of theileriasis.

Nymphae derived from these larvae were fed on 9 August on susceptible Benghazi calf no. 203. This calf developed theileriasis as a result of the feeding, the attack beginning on the 10th day.

Adults from these nymphae were preserved for systematic study.

Summary

(1) The progeny of a single female that had engorged on a reacting calf, with *Theileria* numerous in blood, failed in the larval, nymphal and adult stages to infect susceptible cattle.

(2) *Nymphae* of the second generation, derived from larvae that had fed on a recovered calf, infected a susceptible calf no. 188.

(3) *Adults* of the second generation derived from nymphae, fed during the reaction on an animal suffering from Egyptian fever, transmitted a fatal attack of the disease to a susceptible Benghazi calf no. 185.

(4) *Larvae* of the third generation; the progeny of these adults failed to infect a susceptible calf.

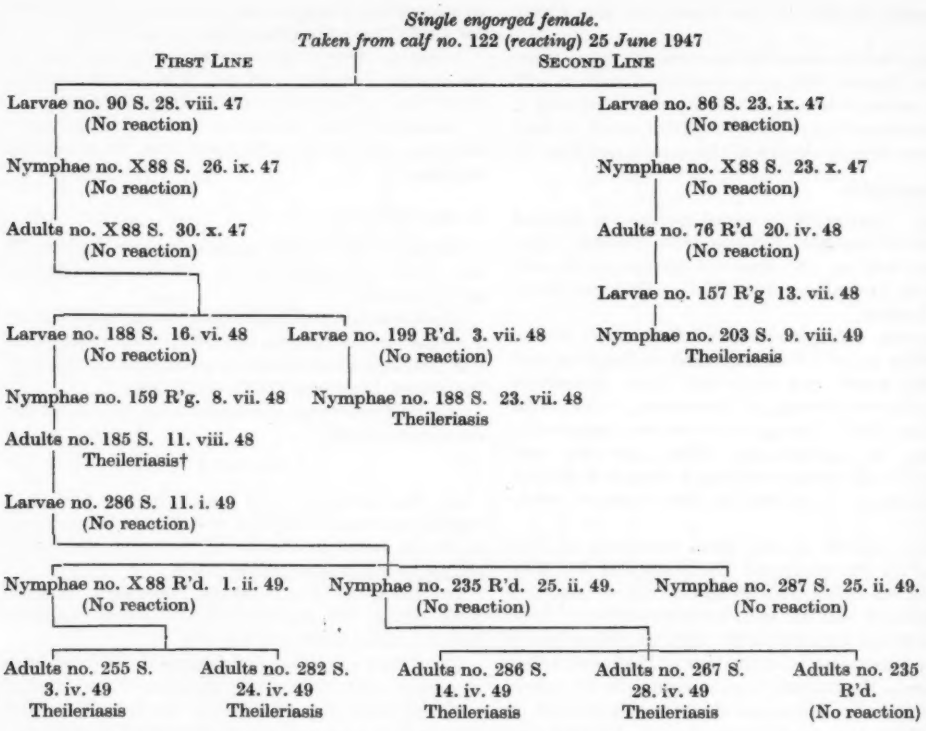
(5) *Adults* derived from nymphae of the third generation, that had been fed on a calf, recovered from an attack of theileriasis 12 months previously, infected two susceptible calves, nos. 255 and 282.

Adults derived from nymphae of the third generation that had fed on a calf recovered from theileriasis 2 months previously, infected two susceptible calves, nos. 286 and 267.

(6) A second line started with larvae from the same engorged female, was also shown to be free from infection through all three instars of the first generation.

(7) *Nymphae* of the second generation, derived from larvae that had fed on a reacting animal, transmitted theileriasis to a susceptible calf, no. 203.

The whole series then includes three separate transmissions by adults infected as nymphae, and two by nymphae infected as larvae. Infection has failed to pass from female to larvae on three occasions, twice when the female was fed on a reacting animal and once when it had fed on a recovered animal.



S., susceptible animal; R'g., reacting animal; R'd., recovered animal.
† Signifies death.

Results obtained with the progeny of the single engaged female tick taken from calf no. 122, suffering from naturally-acquired theileriasis.

Transmission by the inoculation of blood

(1) Susceptible Benghazi calf no. X85 was inoculated on 3 November 1947 with 40 c.c. of citrated blood from reacting Benghazi calf no. 15. The blood at the time was heavily infected with gamonts, agamonts and gametocytes. Calf no. X85 reacted on the 17th day and died of hyper-acute theileriasis 2 days later (see Fig. 6).

(2) Susceptible Benghazi calf no. X89 was inoculated on 23rd November 1947 with 25 c.c. of citrated blood from reacting Benghazi calf no. 50 which was showing gamonts, agamonts and gametocytes in the blood.

Calf no. X89 reacted on the 16th day, developed a mild attack of theileriasis with a few *Theileria* in the blood cells and recovered. The immunity of this calf was subsequently tested on 25 February 1948 by feeding of hibernating adult ticks taken from the wall of the isolation unit. Calf no. X89 did not react to this feeding (see Fig. 7).

THE VECTOR

In 1946, when these investigations were started, species determination in the genus *Hyalomma* was notoriously difficult, and it was for this reason that the series of transmission experiments was undertaken with the second and third generation progeny of a single female. Fortunately the systematic position of the Mediterranean species has now been completely clarified, largely as a result of the persistent efforts made by Delpy (1946*a*, 1949*b*, *c*) in Iran. The work of Adler & Feldman-Muhsam (1946), which directed attention to the morphology of the female genital aperture as an important specific character, was also of the greatest significance in enabling satisfactory species diagnosis to be drawn up.

The present writers have followed Delpy's 1949 classification, and there is no doubt that all the *Hyalomma* that have been collected at the Serum Institute, from buildings and animals, belong to a single species, *H. excavatum* Koch, 1844. The

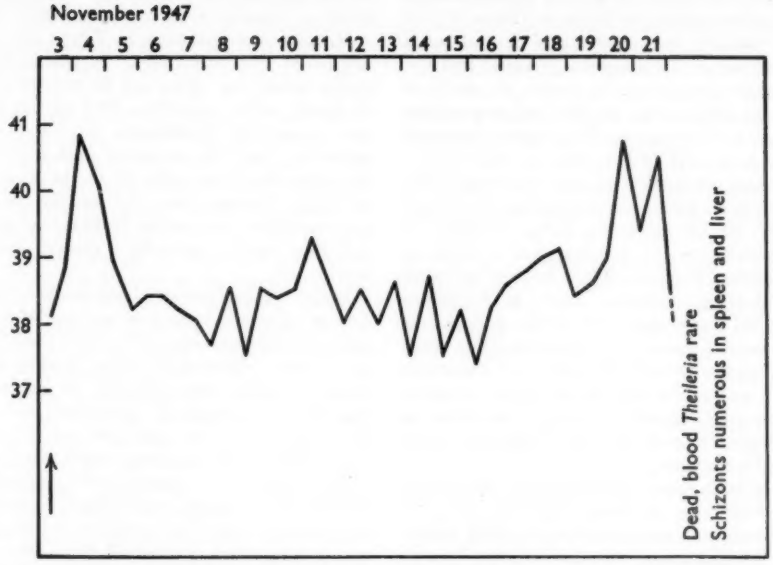

November 1947
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
41
40
39
38
37
Dead, blood Theileria rare
Schizonts numerous in spleen and liver

Fig. 6. Calf no. 85, infected by intravenous injection of 40 c.c. of citrated blood from a reacting calf (no 15).

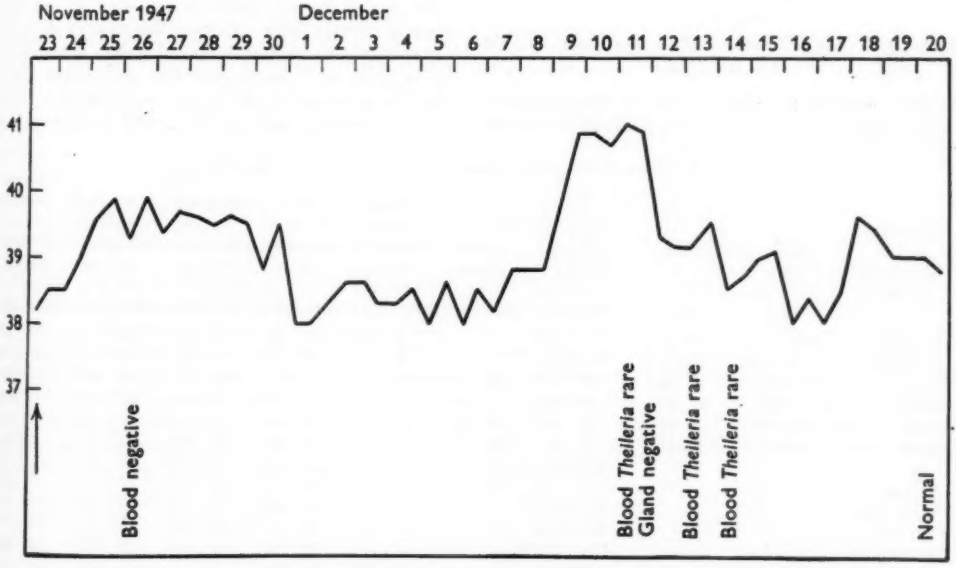


Fig. 7. Calf no. X89, infected by intravenous injection of 25 c.c. of blood from an infected calf (no. 50).

principal characters upon which the determination is based are those utilized in Delpy's (1949*a*) key to the genus, namely, the position of the subanal plates, the extent of the marginal grooves, and the punctation of the scutum in the males; the shape of the genital opening and its papilla, the proportions of the scrotum and the nature of its punctations, and the shape of article II of the palps in the female. These characters are subject to very little variation. The species has an extensive synonymy, for which reference should be made to Delpy (1949*b*, *c*).

According to Delpy, *H. savignyi* (Gerv. 1844), of Adler & Feldman-Muhsam (1947) is identical with his (Delpy's) *H. excavatum*. Adler & Feldman-Muhsam (1946) state that this is the commonest *Hyalomma* species found in Palestine; but they suspect *H. detritum*, which also occurs in Palestine, to be the vector of bovine theileriasis in that country, because it resembles *H. mauretanicum*, the vector in Algeria. Delpy regards *H. mauretanicum* as a synonym of *H. detritum*.

Apparently both these species operate as vectors of bovine theileriasis in Iran, together with *H. savignyi*. Whether *H. excavatum* is the chief vector everywhere in Egypt, or whether *H. detritum* or *H. savignyi* may play an important part in transmission in some parts of this country, is a point that remains to be settled by a survey of the species attacking cattle throughout the territory.

BIOLOGY OF *HYALOMMA EXCAVATUM* IN EGYPT

In the course of the experiments on transmission, observations were made on the general bionomics of the tick under laboratory conditions, and the results of these are condensed in Table 2.

H. excavatum behaves in Egypt as a three-host tick, as it does elsewhere. In one instance only a single larva was observed to remain on the host attached while moulting. The adults feed in the sites usual for *Hyalomma*, i.e. the scrotum, the perineum, and the inguinal and axillary regions. Nymphae feed generally on the neck, chiefly along its crest. Larvae have not so far been taken in large numbers from cattle. Both larvae and nymphae will feed readily from bags placed on the ears of cattle.

Reference has already been made to the hibernation of engorged nymphae and unfed adults in the walls of buildings, and to the fact that adults taken from such hibernating sites during any of the winter months can generally be induced to feed. Engorged hibernating nymphae will not moult during winter unless they are exposed to temperatures higher than ordinary room temperature.

Out of doors it is unlikely that there will be much moulting of nymphae before May, because the nights are usually cold; but possibly a few nymphae in sunny exposures protected from the wind may moult somewhat earlier. The fasting adults do not show any general tendency to leave shelter and attach to the host during the first 3 months of the year, and in any event females that fed abnormally early in the spring would be unlikely to lay until some 2 months later. In late March and April the pre-oviposition period is considerably shortened—to as little as 7 days—and the incubation period may be as short as 27 days. Accordingly, larvae may be feeding early in May, and nymphae from

Table 2. *Bionomics of laboratory-reared* Hyalomma excavatum

Month in which observation started	Room temperature daily range (° C.)		Pre-oviposition interval (days)		Incubation period (days)		First feeding duration (days)		First Ecdysis completion (days)		Second feeding duration (days)		Second Ecdysis completion (days)		Third feeding ♀♀ only duration (days)	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
January	10·5–14·5	12·5–17·5	61*	73*	—		4	9	73	96	7	10	39*	—	9	17
February	9–14·5	14–17·5	No data		—		4	7	18*	21*	5	17	36*	—	8	13
March	11·5–21	13–26·5	10*	18*	35*	46*	5	9	—	—	12	15	20*	25*	25	—
April	14·5–21	17–25	7	21	32	36	—	—	—	—	7	13	20*	31*	6	30
May	21–28	25–32	5	13	22	38	3	8	7	8	5	8	16	19	8	—
June	19–31	28–38·5	3	12	20	24	3	8	8	11	4	10	10	34	—	—
July	—	—	No data		24	24	3	8	7	22	4	8	13	15	—	—
August	—	—	4	6	23	25	3	7	9	9	5	—	15	—	5	10
September	—	—	No data		—	—	4	8	10	23	4	6	10	16	—	—
October	—	—	No data		—	—	—	—	19	—	5	11	33	39	10	17
November	—	—	125*	139*	—	—	—	—	30	43	—	—	38	152	8	23
December	12·5–14·5	14–17·5	96*	108*	—	—	4	10	59	101	—	—	—	—	—	—

* These lots were transferred in the early spring to an incubator room at 30–31° to accelerate development. The minimal period of incubation required to produce oviposition, hatching, or moulting was 10 days and the maximum in the case of hatching of ova 35 days. On the average 14–18 days incubation were necessary to ensure moulting of larvae or nymphal.

late May onwards. Because wild nymphae have been taken engorged from cattle in the month of June, and because the second moult can take place in as little as 10 days during the months June–September inclusive, there seems to be no reason why a second generation should not be completed to the engorged nymphal or fasting adult stage before the end of November. This would give two complete cycles per year. Whether an engorged female can survive the winter in the open and lay in the spring and summer is not known, but engorged females have been observed to survive the winter at room temperatures under laboratory conditions, and to lay viable eggs after a delay of several months. The early incidence of *Theileria* infections in the spring months, shows, however, that the wintering of unfed adults and engorged nymphae is the main method by which the species survives the winter; and it seems likely that the tick could most readily be destroyed by the application of suitable insecticidal agents to the hibernation sites during late winter and spring. Unfed adults do not easily survive the dry summer of Egypt, and when a heavily infested building was kept free from cattle, sheep or goats for a whole year, being used only for poultry, dogs and pigs, no specimens of *Hyalomma excavatum* could be found in the favoured hibernation sites during late summer or during the second winter, and susceptible cattle introduced in the following spring remained free of ticks and did not contract theileriasis. The problem of destroying species that hibernate in buildings seems much simpler than that of controlling ticks, such as *Rhipicephalus* or *Haemaphysalis*, or even the tropical African species of *Hyalomma*, which do not hibernate and which spend the time between feeds widely dispersed on open pastures or bush-veldt.

IDENTITY OF THE *THEILERIA* SPECIES

In Egypt a form of theileriasis, affecting chiefly imported (exogenous) cattle, but capable of giving rise also to clinical infections in Egyptian native cattle has been described or mentioned by Bitter (1905), Mason (1922), Doyle (1924), and Carpano (1937). The causal parasite is readily differentiated from *Theileria* (*Gonderia*) *mutans*, which, in the experience of the senior author, is never even mildly pathogenic for imported animals, and only very rarely evokes parasitic invasions in which a few schizonts may be found. A similar disease has been recorded from Algeria, Palestine, Iraq, Iran, Trans-Caucasia, Southern Russia and India.

Morphologically there is nothing in the descriptions of the *Theileria* found in these infections in the various countries to distinguish the parasites concerned as separate species. Moreover, it has been shown by Delpy (1949*a*) and by Sergent *et al.* (1945), that premunization with the Algerian species, *T. dispar*, affords a marked degree of protection against the Iranian species, and conversely that the Iranian species largely protects against cross-infection with the Algerian parasite. Similarly, there exists a demonstrable degree of cross-protection between the Algerian and Palestine strains (Sergent *et al.* 1945; Adler & Ellenbogen, 1935). There is, in fact, sufficient evidence to warrant an assumption that bovine theileriasis in the Mediterranean countries, Asia Minor and Southern Russia, is caused by a single species of *Theileria*, for which the name *T. annulata* (Dzchunkowsky & Luhz, 1904) should take precedence.

The parasite with which the present paper is concerned differs in no way in morphology, biology or pathogenicity from those described in the other countries mentioned, and may without hesitation be accepted as *T. annulata* (Dzchunkowsky & Luhz, 1904).

SUMMARY

The disease, Egyptian fever of cattle, has been observed at the Serum Institute, Abbassiah, in Cyrenaican and Cyprus cattle, and less frequently in native Egyptian cattle. The responsible *Theileria* has been determined as *T. annulata* (Dzchunkowsky & Luhz, 1904), and both natural and experimental transmission has been demonstrated with *Hyalomma excavatum* Koch 1844, of Delpy (1949*b*).

H. excavatum in Egypt is a three-host tick, which hibernates during the winter months in the walls of buildings in the engorged nymphal and fasting adult stages. Transmission has been effected with nymphae and adults, infected in each case by feeding the preceding stage on reacting or recovered cattle. On no occasion has infection been observed to pass through the egg to the larval stage. Infection can also be transmitted by inoculation of the blood of a reacting animal in which schizonts are demonstrable.

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ON THE MALE OF *ASCAROPHIS MORRHUAE* VAN BENEDEN

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(With 3 Figures in the Text)

Two nematodes, a male and a female, were found in the stomach of a haddock, *Gadus aeglefinus* Linn.; they were lying close together and protruding from the wall of the stomach into its cavity. They were sent to Mr Stephen Prudhoe of the British Museum (N.H.), who identified them as probably *Ascarophis morrhuae* van Beneden. The worms were the only two nematodes found in this fish, and on account of their proximity, and the fact that they both belong to the genus *Ascarophis*, it is assumed that they are a pair. As the male of this species has not apparently been described so far, an account of the single specimen is given below.

The female *A. morrhuae* was originally described and named by van Beneden in 1871, and was the subject of a paper by Nicoll (1907), who referred to it as '*Ascaropsis*' *morrhuae*. It was later re-described by Baylis (1933). The main differences in these accounts appear to lie in the overall lengths of the worms and in the presence or absence of polar filaments on the eggs. The lengths of the two specimens described by Baylis were 11·2 and 13·2 mm. and those by Nicoll 6–8 mm. In the present specimens the length is 4·2 mm. in the male and 5·5 mm. in the female, which sizes approximate those of Nicoll's smaller forms. The eggs (Fig. 2), when they are fully formed *in utero*, measure 44–47 μ (mean 45 μ) by 22–25 μ (mean 24 μ) and each possesses a polar plug bearing two long filaments. Within the uterus, stages in the development of these polar filaments are seen, and the filaments appear to be absent in the more recently formed eggs, but present in the older ones which lie in the region next the vulva. The eggs in this region also show well-developed embryos. The lengths of the filaments, on account of the crowding of the eggs, are difficult to determine, but they are apparently four or five times greater than the length of the egg. Polar filaments were not made out by Baylis, but were observed by van Beneden and by Nicoll. Apart from these differences, the female agrees with the detailed account given by Baylis (1933).

External. The general external appearance of the male is similar to that of the female, both being filiform worms, with similar cuticular striations, and a pair of extremely small anterior labial projections. The cuticular striations become apparent on a level with the beginning of the oesophagus, where they are at first indefinite and lie close together; more posteriorly the distance between them increases and then decreases towards the tail where they are again difficult to observe. In the middle region where they lie farthest apart, the average distance between them is 5·8 μ. Occasional anastomoses occur between adjacent 'rings'.

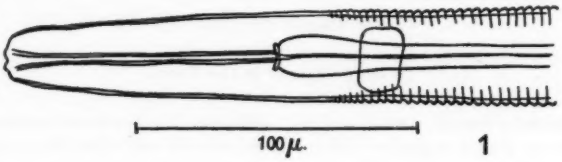
The tail (Fig. 3), curved and spirally twisted, has a bluntly conical extremity ending in a very small papilla. There is a pair of narrow, finely striated, caudal alae, the anterior ends of which lie approximately on a level with the middle of the longer spicule. The posterior region of the worm is slightly hollowed ventrally in front of, and behind, the cloacal opening, and in this region there are four pairs of pre-anal, and five pairs of post-anal pedunculate papillae. A conspicuous longitudinal field (25 μ wide) of well-defined scale-like projections (6·6 × 2·2 μ) lies in front of this hollowed ventral region, extending from a level with the base of the long spicule to just in front of the cloacal opening.

Alimentary canal. The pharynx (Fig. 1), measuring about 0·1 mm. in length, has a wide bore, and its anterior region widens slightly towards the mouth. The oesophagus is relatively very long (1·37 mm.), and is in two parts, a non-granular anterior region of 0·27 mm. and a posterior granular one of 1·10 mm. The anterior, non-granular portion is slightly expanded just behind its junction with the pharynx; it then narrows where it is surrounded by the nerve ring. Posteriorly to the nerve ring it gradually expands to a maximum diameter of 13 μ where it passes into the posterior region. The latter, granular in appearance, has a maximum diameter of 30 μ and almost fills the body cavity.

Reproductive system. The single testis has its origin close to the junction of the oesophagus and the intestine, and just before the tail region the vas deferens shows a constriction, presumably differentiating the duct from the seminal vesicle.

The spicules are of very unequal length (right spicule 90 μ and left 590 μ). Both are, however, of uniform diameter throughout the greater part of the length, but taper towards the apex and are hollowed towards the base along one border (Fig. 3).

There is some little doubt as to the identity of the specimens here described, because of the length of the female and the presence of polar filaments on the eggs. In the original description of *Ascarophis morrhuae* van Beneden refers to 'sa petite taille' but the larger worms described by Baylis, which were worms in which polar filaments were not observed on the eggs, may be specifically distinct. The female nematode identified here is only slightly smaller than the worms recorded by Nicoll, and in the



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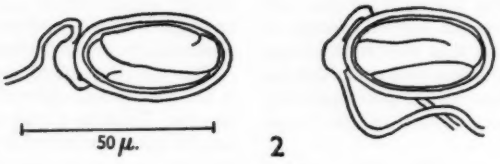

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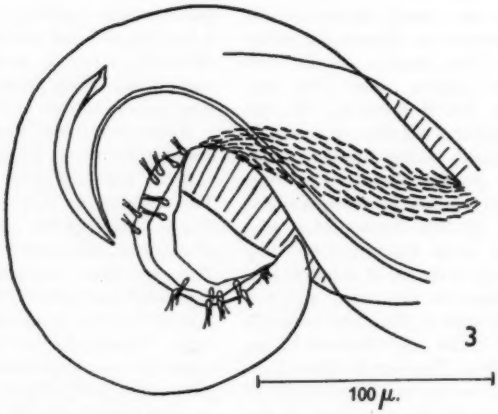

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Fig. 1. *Ascarophis morrhuae*, anterior end of the male.
Fig. 2. *Ascarophis morrhuae*, Uterine ova showing the origin of the polar filaments and the contained embryos.
Fig. 3. *Ascarophis morrhuae*, posterior end of the male.

gives no measurements. Nicoll (1907) refers his worms to the same species, and these measured 6–8 mm. Although the worms described by Baylis (1933) were almost twice the size of Nicoll's specimens, they may also be the same species, because a great variation in length is recorded in many nematodes. On the other hand, it is possible that absence of further differences it is accordingly referred to the species of van Beneden *A. morrhuae*.

My thanks are due to Mr D. R. R. Burt for his invaluable assistance and advice, and to Mr Prudhoe of the British Museum for his helpful criticism.

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A STUDY OF THE VECTOR-PARASITE RELATIONSHIPS IN *SCHISTOSOMA MANSONI*

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(With 1 Figure in the Text)

In an earlier paper (Files & Cram, 1949) striking differences in susceptibility of snail vectors for various strains of *Schistosoma mansoni* were reported. It was noted that in general *Australorbis glabratus* from Puerto Rico and Venezuela and *Biomphalaria pfeifferi* from Liberia were more favourable vectors for the various strains of *Schistosoma mansoni* than *Australorbis glabratus* from Brazil or *Biomphalaria boissyi* from Egypt. In the present paper a series of experiments are reported which were designed to investigate further the differences in the susceptibility of these and other vectors, and to suggest what factors may be responsible for these differences.

MATERIALS AND METHODS

Australorbis glabratus from Puerto Rico, the Dominican Republic, Venezuela, Surinam, and two areas in Brazil (Recife and Salvador), *Biomphalaria pfeifferi* from Liberia and *B. boissyi* from Egypt were employed in this study. The *Australorbis glabratus* were obtained from laboratory-reared colonies established from eggs or adult specimens of the snail vector that had been received from the following sources: Puerto Rico, four lots from San Juan, 1943, 1946, 1947, 1949; Dominican Republic, two lots from Ciudad Trujillo, 1949; Venezuela, one lot from Caracas, 1946, and one lot from Lake Valencia, 1947; Surinam, four lots, two from Paramaribo and two from Kawatta, 1949; Brazil, four lots, two from Recife, 1946 and 1947, and two lots from Salvador, 1949. *Biomphalaria pfeifferi* were drawn from colonies established from adult specimens received from Monrovia, Liberia, 1948 and 1949. *B. boissyi* were obtained from colonies established from adult specimens received from Cairo, Egypt, 1947 and 1949. All three species of snails were identified by Dr Elmer G. Berry of this laboratory.

Schistosoma mansoni used in these experiments was obtained from infected animals shipped directly from various endemic areas or was established in laboratory animals from infected snail vectors received from the endemic areas. In a few instances *S. mansoni* of human source was employed. *S. mansoni* from a specific geographical area, such as Puerto Rico or Venezuela, is referred to as a 'strain' of the parasite. The following five strains of *S. mansoni* were used for the exposure of the snails: Puerto Rican, Venezuelan, Surinam, Brazilian and Egyptian.

In addition to the five strains of *S. mansoni* just mentioned, seven other strains of parasite were established by the crossing of certain of the regular strains. The development of interstrain crosses was found to be necessary because of a lack of pure strains of the parasite from some endemic areas. For example, several *Australorbis glabratus* from Recife, Brazil, were received in the laboratory. However, only one specimen shed cercariae and these developed in laboratory animals into female schistosomes only. In order to secure ova, animals exposed to the cercariae which developed into female worms were also exposed to cercariae of the Puerto Rican strain which were known to develop into male worms only. Thus a cross-strain, hereafter referred to as Recife cross-strain, was established.

A guinea-pig infected with *Schistosoma mansoni* from Salvador, Brazil, was received in the laboratory. Miracidia from this animal infected Puerto Rican *Australorbis glabratus*, but again only female schistosomes were recovered from animals exposed to cercariae from these infected snails. Unfortunately, the guinea-pig died, and we were unable to infect additional snails with this Brazilian strain. Animals infected with these female schistosomes were then exposed to cercariae known to develop into male schistosomes only. These male worms were from the following strains: Puerto Rican, Egyptian, and the Recife cross, respectively. These last cross-strains are referred to as the Salvador crosses.

In the same manner three Egyptian cross-strains were developed: Egyptian females × Puerto Rican males, Egyptian females × Venezuelan males, and Recife-cross females × Egyptian males (see Table 1).

The method of exposing the snails was essentially the same as that reported previously (Files & Cram, 1949). Miracidia were obtained from liver and faeces of mice and hamsters, monkey faeces, or human faeces. Only freshly hatched miracidia were used. In the majority of the experiments the snails were exposed individually to ten miracidia each, but in a few instances several hundred miracidia were

* With the technical assistance of Rodney H. Duvall.

employed in an attempt to infect apparently refractory snails by massive exposure. The exposures were performed in small syracuse dishes; snails were kept in these for approximately 12 hr. in order to assure ample time for penetration. Tests for susceptibility were done in a series of small experiments, each experiment involving from ten to fifty snails. Therefore, in most cases, the total number of snails exposed to any specific strain of *Schistosoma mansoni*, as recorded in the table, represents the aggregate of several experiments. In a few instances, because of an insufficient number of animals infected with a particular strain of parasite, it was possible to test only a single small group of snails. In addition, it was not always possible to expose snails of all the geographical groups to all the strains of *S. mansoni* because the supply of snails or miracidia was often limited.

Table 1. *Crosses of* Schistosoma mansoni *from different geographic localities to produce new cross-strains of the parasite*

Designation of cross-strain	Origin of female schistosome of the cross	Origin of male schistosome of the cross
Recife cross	Recife	Puerto Rico
Salvador cross 1	Salvador	Puerto Rico
Salvador cross 2	Salvador	Recife cross
Salvador cross 3	Salvador	Egypt
Egyptian cross 1	Egypt	Puerto Rico
Egyptian cross 2	Egypt	Venezuela
Egyptian cross 3	Recife cross	Egypt

Exposed snails were maintained in small aquaria containing greenery and gravel, were fed lettuce and fish food daily, and kept at a temperature of 24° C. Upon completion of the prepatent period, at approximately 28–30 days, all specimens were isolated in test-tubes and checked for the emergence of cercariae. Isolations were continued at intervals up to the fiftieth day. Snails which died during the observation period were dissected to determine whether there was any development of the parasite.

The percentage of snails shedding cercariae within 50 days of exposure was used as a criterion of susceptibility. In these experiments, snails showing only sporocyst development were not labelled as 'infected'. Infectivity rates below 20 % were considered indicative of a low degree of susceptibility; infectivity rates above 60 % were considered indicative of a high degree of susceptibility; infectivity rates between 20 % and 60 % were considered as indicative of a moderate degree of susceptibility.

RESULTS

A total of 3046 *Australorbis glabratus*, 442 *Biomphalaria pfeifferi*, and 371 *B. boissyi* was exposed to the various strains of *Schistosoma mansoni*. The results of these exposures are summarized in Table 2.

Table 2. *Results following the exposure of* Australorbis glabratus, Biomphalaria pfeifferi *and* B. boissyi *to various strains of* Schistosoma mansoni

Strains and source of *S. mansoni*	*A. glabratus* from Puerto Rico		*A. glabratus* from Venezuela		*A. glabratus* from Dominican Republic		*A. glabratus* from Surinam		*A. glabratus* from Brazil (Recife)		*A. glabratus* from Brazil (Salvador)		*B. pfeifferi* from Liberia		*B. boissyi* from Egypt	
	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T
Puerto Rican (A)*	54	(207)	42	(112)	—	—	82	(39)	0	(132)	0	(32)	76	(121)	0	(43)
Puerto Rican (H)*	45	(94)	20	(20)	—	—	30	(37)	7	(88)	0	(130)	—	—	0	(30)
Venezuelan (A)	53	(127)	32	(87)	27	(22)	—	—	0	(110)	—	—	97	(48)	0	(50)
Surinam (A)	36	(19)	—	—	—	—	46	(82)	—	—	0	(67)	66	(74)	—	—
Surinam (H)	—	—	—	—	—	—	—	—	—	—	0	(100)	—	—	—	—
Brazilian (Salvador) (A)	6	(50)	—	—	—	—	—	—	0	(49)	—	—	—	—	—	—
Egyptian (A)	12	(134)	8	(87)	0	12)	14	(42)	0	(107)	0	(76)	30	(35)	31	(94)
Recife cross (A)	40	(104)	54	(84)	25	(38)	40	(45)	52	(132)	0	(24)	42	(21)	0	(73)
Salvador cross 1(A)	73	(30)	—	—	—	—	62	(29)	—	—	0	(85)	—	—	—	—
Salvador cross 2 (A)	44	(18)	—	—	—	—	38	(67)	—	—	0	(136)	100	(32)	—	—
Salvador cross 3 (A)	88	(36)	—	—	—	—	—	—	—	—	0	(47)	14	(35)	0	(63)
Egyptian cross 1 (A)	34	(29)	—	—	—	—	—	—	—	—	0	(19)	96	(56)	3	(33)
Egyptian cross 2 (A)	31	(16)	—	—	—	—	—	—	—	—	—	—	—	—	*0	(17)
Egyptian cross 3 (A)	36	(38)	—	—	—	—	—	—	—	—	—	—	—	—	0	(31)

%=percentage of specimens positive for *S. mansoni*.
Numbers in parentheses=total number of specimens remaining at the end of prepatent period, i.e. 28–30 days after exposure.
(A)*=*S. mansoni* from animal source: mice, hamsters and monkeys.
(H)*=*S. mansoni* from human faecal samples.
*0=Secondary sporocysts recovered from two specimens.

Puerto Rican *Australorbis glabratus* became infected with all the strains of *Schistosoma mansoni*; it showed a moderate to high rate of infection with the Puerto Rican, Venezuelan, Surinam, the Recife cross, the Salvador crosses, and the Egyptian cross-strains and a low rate of infection with the Brazilian and Egyptian strains.

Venezuelan *Australorbis glabratus* evidenced a moderate rate of infection with the Puerto Rican, Venezuelan, and Recife cross-strain, and a low rate of infection with the Egyptian strain.

Dominican Republic *A. glabratus* became moder-

ately infected with the Venezuelan and the Recife cross-strain but was refractory to the Egyptian strain.

Surinam *A. glabratus* showed a relatively moderate to high infectivity rate when the Puerto Rican, Surinam, the Recife cross and two Salvador cross-strains were employed, but showed a low infectivity rate when the Egyptian strain was used.

Brazilian *A. glabratus* from Recife, however, could not be infected with the Venezuelan, Egyptian and Brazilian strains, and gave only a low infectivity rate with the Puerto Rican strain of human source. On the other hand, these snails became moderately infected with the Recife cross-strain.

Brazilian *A. glabratus* from Salvador proved refractory to all the strains of parasite tested, even to the Salvador cross-strains.

Liberian *Biomphalaria pfeifferi* showed a high degree of receptivity for the Puerto Rican, Venezuelan, one Salvador cross, and one Egyptian cross-strain, and a moderate to low degree of receptivity for the Recife cross, one Salvador cross, and the Egyptian strain.

Egyptian *B. boissyi* was refractory to the Puerto Rican, Venezuelan, the Recife cross, one Salvador cross, and two Egyptian cross-strains. However, these snails could be moderately infected with the Egyptian strain and, in addition, one specimen shed cercariae after being exposed to the Egyptian cross, Egyptian × Puerto Rican, and partial development of the parasite was noted in two snails that had been exposed to the Egyptian cross, Egyptian × Venezuelan.

DISCUSSION

To summarize the results briefly it may be said that: *Australorbis glabratus* from Puerto Rico, Venezuela, the Dominican Republic and Surinam were more susceptible to strains of *Schistosoma mansoni* from the Western Hemisphere than to the strain of *S. mansoni* from Egypt. In contrast, *Australorbis glabratus* from Brazil was refractory to almost all the strains of *Schistosoma mansoni* employed in this study. The snails from Recife could only be readily infected with the Recife cross-strain, while the snails from Salvador were refractory to all the parasite strains used.

Biomphalaria pfeifferi from Liberia was a suitable host for the strains of *Schistosoma mansoni* from the Western Hemisphere and for the Egyptian strain. On the other hand, *Biomphalaria boissyi* from Egypt was receptive only to the Egyptian strain and one Egyptian cross-strain.

It is clear that, in general, the snails were highly susceptible to infection with schistosomes of their own endemic areas but in some cases quite refractory to parasites from other endemic areas. This compatibility of host and parasite from a given endemic area is to be expected since the life cycle is maintained in that area. The relative incompatibility between snail and parasite from different endemic areas calls for additional explanation. While in the present state of our knowledge no fully adequate explanation can be offered, a discussion of some of the biological and historical factors which may be involved is of considerable interest.

From this study it appears that there are interspecific and intraspecific differences between vectors in regard to the physiological factors that determine the development or inhibition of the parasite in the snail. It is evident that the three species, *Australorbis glabratus*, *Biomphalaria pfeifferi*, and *B. boissyi* differ in their susceptibility for the several strains of *Schistosoma mansoni*. In addition, present evidence indicates that vectors of the same species but of different geographical areas may differ as regard these factors. Such differences are exhibited by the *Australorbis glabratus* from Puerto Rico, Venezuela, the Dominican Republic, and Surinam, on the one hand, and the *A. glabratus* from Brazil, on the other hand. Since the snails of all these areas have been identified as the same species, namely, *A. glabratus* (Say), then the difference in susceptibility of these groups of snails must be due to intraspecific physiological differences.

However, differences between vectors cannot alone explain the incompatibility between snail and parasite of different endemic areas. Physiological differences in the parasites of different endemic areas also are apparent. For example, it has been noted that *A. glabratus* from Recife was refractory to several strains of *Schistosoma mansoni* and, in the absence of the true Recife strain, could only be readily infected with the Recife cross-strain of parasite. Since these snails were refractory to some strains of *S. mansoni* but were highly susceptible to the Recife cross-strain of *S. mansoni*, it may be concluded that this latter strain differs in some manner from the former strains.

It will also be noted that the parasite of Egypt differs from the parasites of the Western Hemisphere employed in these experiments. *Biomphalaria boissyi* could not be infected with *Schistosoma mansoni* from Puerto Rico, Venezuela, or Surinam. However, these snails were susceptible to the Egyptian strain of *S. mansoni*; infection was also obtained on one occasion with one Egyptian cross-strain of parasite, the Egyptian × Puerto Rican cross-strain. In addition, partial development of the parasite, i.e. secondary sporocysts, was obtained when the Egyptian × Venezuelan cross-strain was employed. This partial development of the parasite is significant, for in no case could such development be demonstrated when the snails were exposed to strains of *S. mansoni* of the Western Hemisphere. It would appear, therefore, that the Egyptian parasite differs

in some manner from the parasite of Puerto Rico, Venezuela and Surinam used in this study. The limited susceptibility of *Australorbis glabratus* from Puerto Rico, the Dominican Republic, Venezuela and Surinam for the Egyptian strain of *Schistosoma mansoni* is additional evidence for such a difference.

A more detailed explanation of the susceptibility of the vectors for the cross-strains of parasite may be of interest. As has been noted, it was possible to infect certain of the relatively refractory vectors with cross-strains of the parasite, one sex of which was derived from the strain of *S. mansoni* from the vector's own endemic area and the other sex of which was derived from a 'foreign' strain. Thus, the *Australorbis glabratus* from Recife could be infected with the cross-strain of Recife females and Puerto Rican males; *Biomphalaria boissyi* could be infected with the cross-strain of Egyptian females and Puerto Rican males and, in addition, partial development of the parasite was obtained with the cross-strain of Egyptian females and Venezuelan males. On the other hand, *B. boissyi* could not be infected with the cross-strain of Recife-cross females and Egyptian males or the cross-strain of Salvador females and Egyptian males. It is interesting to note that successful infection with the cross-strains of *Schistosoma mansoni* has only occurred in those instances where the female schistosomes, and not the male schistosomes, were derived from the strain of parasite of the vector's own endemic area. This would suggest that the factor or factors that characterize 'strain' susceptibility were contributed by the female schistosomes in these cases. The failure to infect *Australorbis glabratus* from Salvador with corresponding cross-strains of Salvador females and male schistosomes of Puerto Rico, the Recife cross and Egypt, respectively, cannot be explained.

The compatibilities and incompatibilities between vectors and parasites of different endemic areas give some further suggestions as to the possible epidemiology of *Schistosoma mansoni*. The present evidence indicates that in the Western Hemisphere the vectors and parasites of Puerto Rico, Venezuela and Surinam are physiologically similar, while the vector and parasite of Brazil are physiologically different from those of the former countries. No explanation can be offered for the differences between vectors, but there are two alternative explanations for the development of physiologically distinct strains of parasite: first, the strain of parasite established in Brazil was originally different from the strain of parasite established in some of the other countries of the Western Hemisphere; or secondly, the same strains of parasite were established in all the countries of the Western Hemisphere but in some areas, namely Brazil, the parasite became modified.

It has been generally assumed that *S. mansoni* was brought to the Western Hemisphere when slaves were introduced. Thus, it is possible that the *S. mansoni* established in Brazil was derived from a different geographical area in Africa than the *S. mansoni* established in other parts of the Western Hemisphere. From the records of the slave trade some information is available about the origin of the slaves introduced into the West Indies and Brazil (Fig. 1). As regards the West Indies, Montagu (1944) states that the evidence indicates that negroes were already established on the island of Hispaniola in 1502. These first slaves were imported from Spain and their origin in Africa is rather obscure. With an increase in the demand for labourers, however, slaves were soon being imported directly from the west coast of Africa to the West Indies and North America. It is generally agreed by Montagu (1944) and Herskovitz (1941) that these later slaves were drawn principally from the coastal areas of West Africa, from Senegal in the north to Angola in the south. Herskovitz presents the records from five brokerage firms dealing in slaves that give the origin of slaves imported into the British West Indies for the years 1764–74, 1782–8, 1779–88, and 1786–8*. Of over 34,000 slaves imported, 10,000 were from the Niger Delta region of Nigeria, 14,000 from the Gold Coast, 4000 from Togo and Dahomey, 2500 from the Windward coast, and the remaining number from scattered areas on the west coast of Africa.

Ramos (1939) presents information about the origin of the slaves imported into Brazil. He states that there were three negro peoples that entered Brazil: (1) the Sudanese from the Gold Coast and Dahomey area, (2) the Moslem negroes from the Guinea and Senegal area, (3) the Bantus from the Congo, Angola and Mozambique. Thus, one group of slaves, the Sudanese, correspond to the slaves reported to have been imported into the British West Indies. However, the other two groups of negroes entering Brazil were from north-west and central Africa, as opposed to middle-west Africa. It is not possible to determine which of the various groups of slaves were responsible for the establishment of schistosomiasis in Brazil, but it is interesting to note that there was quite a diversified group of negroes that entered and possibly one group of these slaves introduced *S. mansoni*. For example, Ramos remarks that 'Cabindas, from the Congo, Angolas, Benuelas and a small number of Sudanese were the slaves responsible for the development of the sugar industry in north-east Brazil' (p. 13). Since *S. mansoni* is not endemic in Angola and only a few Sudanese slaves were used in the sugar industry, it would seem that the Cabindas and the Benguelas, both of central Africa,

* According to the information supplied by the British Embassy, The British West Indies from the years 1764 to 1788 were comprised of Jamaica, Barbados, Antigua and St Kitts.

might have been responsible for the establishment of *S. mansoni* in certain of the endemic areas in north-east Brazil.

It is possible, therefore, that a different strain of *S. mansoni* was introduced into Brazil than the strain of parasite introduced into the West Indies and surrounding islands. However, a second explanation could be that the parasite of Brazil became modified after being established in that country. It is not possible to determine what factors could be responsible for such modification. Perhaps the parasite and vector of this area became relatively isolated as compared to the parasite and vector of other endemic areas, and such isolation resulted in physiological changes in the parasite. Thus, it might be that local races and strains of *S. mansoni* have evolved in certain endemic areas. Actually, the point under discussion here is merely a matter of where the modification arose, in Africa or in the New World.

It is suggested that *S. mansoni* originated in East Africa, probably in Egypt, where the disease is known to have existed in 1000 B.C., and that from here it became disseminated throughout Africa, and was finally brought to the Western Hemisphere from West Africa. It is further suggested that in certain endemic areas local races or strains of *S. mansoni* have evolved, so that the parasites of various geographical areas may differ physiologically. From this study it appears that the *S. mansoni* endemic in Brazil is physiologically distinct from the *S. mansoni* endemic in many other parts of the Western Hemisphere; *S. mansoni* of Egypt is physiologically distinct from the *S. mansoni* of the Western Hemisphere. As regards the vectors (Fig. 1) *Australorbis glabratus* from Puerto Rico, Venezuela and Surinam and *Biomphalaria pfeifferi* from Liberia are apparently versatile vectors, serving as host for several strains of *Schistoma mansoni*, while *Australorbis glabratus* from Brazil and *Biomphalaria boissyi* from Egypt are less versatile, serving as hosts for the strains of *Schistosoma mansoni* from their own endemic areas, but being refractory to the other strains of parasite employed in this study.

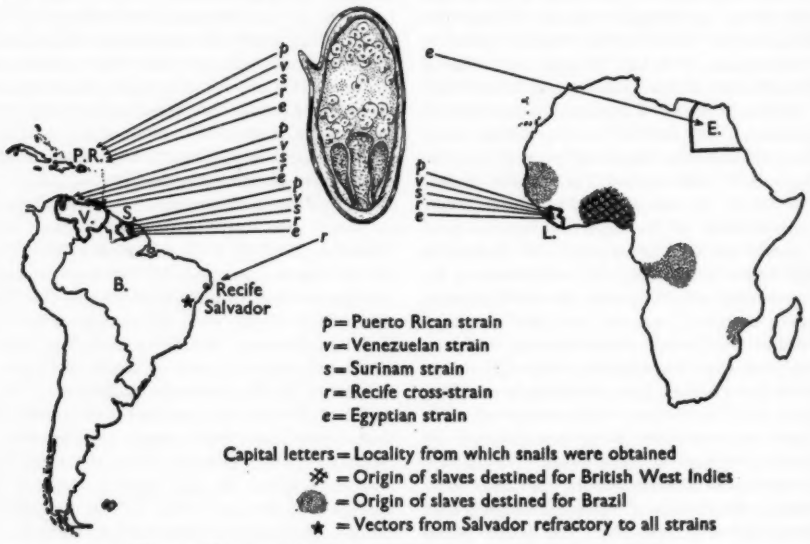

Fig. 1. The susceptibility of vectors for strains of *S. mansoni*.

SUMMARY

In this study *Australorbis glabratus* from Puerto Rico, the Dominican Republic, Venezuela, Surinam and Brazil, *Biomphalaria pfeifferi* from Liberia, and *B. boissyi* from Egypt were exposed to strains of *Schistosoma mansoni* from Puerto Rico, Venezuela, Surinam and Egypt, and to seven cross-strains of *S. mansoni*. In many instances, incompatibilities between vectors and parasites of different endemic areas were noted. It is suggested that these incompatibilities might be accounted for in two ways: (1) interspecific and intraspecific differences between vectors in respect to the physiological factors that are responsible for the development of the parasite in the snail, and (2) physiological differences between strains of the parasite. Evidence would seem to show that there are interspecific physiological differences between *Australorbis glabratus* and *Biomphalaria boissyi*; and that there are intraspecific

differences between *Australorbis glabratus* from Brazil and *A. glabratus* from other countries here dealt with. In addition, it would appear that the parasite of Egypt differs physiologically from the parasites of Puerto Rico, Venezuela and Surinam; and that the parasite of Brazil differs physiologically from the parasites of the latter countries.

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DESCRIPTION OF A PLASMODIUM FOUND IN A BAT, *ROUSSETTUS LEACHI*

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(With Plate IV)

I. INTRODUCTION

Haemosporidea have been known in bats for over half a century, but only a few papers are to be found on the subject. The work of Danilewski, of MacCallum, of Roehl, of James and Tate and of others upon avian malaria, as well as the successful researches upon simian malaria by J. Rodhain, amongst others, have played an important part in furthering our knowledge of malaria. Studies on the comparative parasitology of the plasmodia of the Chiroptera might increase our knowledge still further, especially with regard to epidemiology, pathogenicity and therapeutics. The breeding of the insectivorous Microchiroptera proves difficult in the laboratory, but the large frugivorous Chiroptera are more likely to prove suitable for experimentation.

During recent years, the possibilities of experimental malariology have been greatly extended by the important discovery by Vincke & Lips (1948) of *Plasmodium berghei* in *Thamnomys surdaster surdaster*. This plasmodium is easily transmitted to other rodents, including the white mouse in which it produces a very acute infection. However, in spite of the extreme interest of such researches, they do not exhaust the subject; the study of infections of mammals with other plasmodia and their experimental transmission would widen the field still further.

Our knowledge of chiropteran malaria is small, and the mode of transmission of these parasites is unknown. The part played by Nycteribiidae and Streblidae is, as yet, only an hypothesis. We know little of their geographic distribution, of their cycle of development and of the reaction of the vertebrate hosts. Doubts have been entertained about their classification among the Plasmodidae. Although incomplete, the data obtained so far give the impression that there are numerous species of parasites. For comparison with our own observations, we shall summarize the characteristics of the three species which can at present be considered as valid.

The malaria parasite of the Chiroptera was discovered by Dionisi (1899*a*, *b*) who at first thought that he had found in these animals the three species known in man, but he soon had to abandon this belief. One of the species which he described proved to be a Piroplasma, and the distinction between the two others, based on differences in the pigment in the gametocytes, cannot be retained as valid. In fact, there is only one pigmented species, which Dionisi named *Plasmodium melanipherum*; *Miniopterus schreibersii* and *Vespertilio murinus* were the first hosts to be described. Most of the parasites found in the blood were gametocytes, and Dionisi did not observe any segmenters. The same parasite was identified in other Vespertilioninae, in Italy by Sambon & Low (1901) in *Myotis capaccini*, and in South Africa by Bowhill (1906) in *Vespertilio capensis*. Shingareff (1907) rediscovered it in Russia in two species of bats, *V. daubentoni* and *Miniopterus schreibersii* (the latter had already been found by Dionisi to be parasitized). In the latter, practically only gametocytes were found, but in *Vespertilio murinus* Shingareff observed segmenting forms, rare in peripheral blood, but numerous in the liver and spleen, where some were found lying in the leucocytes whereas others were free.

The parasite found by Vassal (1907) in *Vesperugo abramus*, in Annam, was probably also *Plasmodium melanipherum*, as was that discovered by Coles in *Vesperugo pipistrellus* in England (1914–15), and by de Mello & Braz de Sâ (1916) in *Vespertilio muricola* in the Portuguese Indies (although the latter might have been a Piroplasma), in *Eptesicus* in South Africa (quoted by Allen, 1939), by McGhee (1949) in *Miniopterus australis* in the New Hebrides, and by Mer & Goldblum (1947) in *Myotis myotis* and other species in Palestine. The last-named authors describe an intense process of schizogony taking place in the reticulocytes and the granulocytes of the bone marrow. Exo-erythrocytic forms were also present in the free and fixed macrophages of the lung, kidney and liver.

Plasmodium melanipherum is the only species of plasmodium described from the suborder of the insectivorous Microchiroptera. Among the Megachiroptera two species have been determined with certainty: *P. pteropi* and *P. epomophori*.

In 1908, Durham saw a plasmodium in the blood of *Pteropus natalis*, in Christmas Island. In 1911, Breinl proposed the name of *P. pteropi* for the

parasite he observed in Australia in *P. gouldi*. He described a fragmentation of the chromatin into a dozen small blocks, but no true rosettes were observed. Other species of bats of the same genus were subsequently found parasitized, e.g. *Pteropus edwardsii* in India (Mackie, 1914), and *P. medius* at the London Zoological Garden (Scott, 1927). Finally, in 1946, Manwell gave a precise description, illustrated with photo-micrographs, of a parasite he observed in material sent from New Guinea, and found in *P. gouldi*. The American parasitologist, taking as his criterion the absence of segmenters in the peripheral blood, deems it probable that all the intra-erythrocytic forms give rise to gametocytes. In a few blood smears he found schizonts and apigmented, extracellular forms undergoing division which look extraordinarily like the exo-erythrocytic forms of avian plasmodia. This stage was not seen in the liver, bone marrow, lungs or spleen. Quoting Garnham (1948): 'it is possible that these bodies in reality were detached portions of a rupturing merocyst', and 'it is doubtful whether they should be regarded as the mammalian equivalent of *Plasmodium elongatum* because, although in the blood, they lie free and are not in cells of the haemopoïetic system'. McGhee (1949), in the Philippines, did not see the exo-erythrocytic forms described by Manwell and did not observe any stage of division of *P. pteropi*, although it was present in all the bats examined which belonged to the species *Pteropus geddiei* and *P. colinus*.

The third species, *Plasmodium epomophori*, presents, for those who observed it, a characteristic morphology. Rodhain (1915, 1926*a*, *b*) studied it in the Belgian Congo in four species of bat, *Epomophorus wahlbergi haldemani*, *E. franqueti franqueti*, *Hypsognathus monstruosus* and *Micropterus pusillus*. He did not find any rosettes, but saw some pre-segmenters with six to eight chromatin blocks. Attempts at transmission by *Stegomya fasciata*, Culicinae and *Cimex lectularius* did not meet with any success, but he succeeded in infecting other bats by direct inoculation of parasitized blood. *Plasmodium epomopori* was also observed by A. & M. Leger (1914) in the Senegal, in *Epomophorus gambianus*.

Beyond these three well-characterized species, plasmodia were found in other types of bats.

Among the insectivorous bats, two more species were found parasitized, e.g. *Nycteris hispida* in the Belgian Congo (Rodhain, 1926*a*, *b*) and *N. grandis* in Liberia (Theiler, 1930). In Italy, *Rhinolophus ferrum-equinum* also is infected with a plasmodium (Moriggi, 1940). As regards the Megachiroptera, Donovan (1919–20) noted the presence of a haemosporida in a 'Fijian flying fox' and Manwell (1946) found the gametocytes of a plasmodium in the blood of *Dobsonia moluccensis* in New Guinea.

II. MATERIAL

The following description is of a plasmodium found by us in *Roussettus leachi* A. Smith. We have described elsewhere (J. Van Riel, D. & J. Hiernaux, 1948) the results of a zoological and parasitological investigation carried out from 10 to 18 August 1947 in the caves of Mount Hoyo (Belgian Congo). Forty-one males and twenty-two females of *R. leachi* were captured. As the bats were hanging down from the high ceiling of the caves, we shot them at first with a gun. The animals so captured had little chance of survival. However, two survived for a fairly long period and were fed mainly with bananas.

A second trip to the caves was made from 23 to 26 November 1948. Thirteen roussettes were captured; they were sent to the Institut de Médecine Tropicale at Antwerp, where further studies of their parasites were to be undertaken, but owing to various misfortunes, six only arrived there alive.

Finally, in the course of a third expedition, from 16 to 18 April 1948, twenty-seven roussettes were captured with a net and brought back to camp in individual cages, in the hope of diminishing the percentage of deaths. However, even so, only eight arrived alive in Antwerp.

The important material collected from living or dead animals (blood-smears, contact smears of organs, etc.) enabled us to identify in *R. leachi* a plasmodium which has been studied both on the spot in Africa, and in Europe at the Institut de Médecine Tropicale in Antwerp.

Out of the eighty-one bats examined, we found seven parasitized, i.e. about 9 %; but, as will be seen later, latent submicroscopic infections might well be more frequent.

III. DESCRIPTION OF THE PARASITE

In stained preparations the young trophozoite shows a red nucleus and a slender mass of blue cytoplasm without pigment. Sometimes the parasite seems to lie at the periphery of the red blood corpuscle. Soon, a vacuole appears, and the nucleus is then situated in the cytoplasmic ring around it, and in some cases protrudes into the vacuole (Pl. IV, fig. 1). A supplementary chromatin granule is sometimes to be found in the cytoplasm. Dark brown pigment appears as the vacuole develops.

At first the ring fills only one-quarter of the erythrocyte in which it lies, but it rapidly grows and may fill practically the whole of it. The persistence of the ring-shape up to an advanced stage of development is a feature which is rather specific to this parasite. Large rings have been observed lying apparently free in the cytoplasm (Pl. IV, fig. 2). The regular outline of the trophozoites indicates weak amoeboid movement or its absence.

The schizonts are rounded or polygonal (Pl. IV, fig. 3). The nucleus divides several times in succession. When only four nuclear masses are present (Pl. IV, fig. 4) the pigment granules are still scattered, but in the typical rosettes with eight merozoites (Pl. IV, fig. 5), where each of the daughter nuclei is surrounded with cytoplasm, the pigment is aggregated into one central block. Up to three rosettes have been observed in one microscopic field (Pl. IV, fig. 6).

The young gametocytes differ from the schizonts by the absence of the vacuole. They invade the whole erythrocyte progressively until it can only be seen as a thin border or not at all. Adult gametocytes are spherical, ovoid or polygonal.

Macrogametes, stained with Giemsa's stain, have a purplish blue cytoplasm. The round or kidney-shaped nucleus is small and dense. It may be centrally (Pl. IV, fig. 7) or peripherally situated. The pigment is scattered throughout the whole cytoplasm; although there is usually a clear zone round the nucleus.

The cytoplasm of the microgametocyte is less chromatophilic than that of the macrogametocyte. It may be so colourless as to be barely distinguishable against the background of the erythrocyte. The nucleus is either centrally or peripherally placed (Pl. IV, fig. 8) and is larger and more diffuse than in the female gametocyte. Its outline is vague, its shape triangular, oval or polygonal. The pigment is either scattered uniformly with the exception of a clear perinuclear zone, or clumped in large blocks which form a ring at the periphery.

The parasitized erythrocytes are normal in size and do not contain any granulations, but often show a basophilic staining. An erythrocyte rarely contains more than one parasite.

IV. DEVELOPMENT OF THE PARASITE

During our first investigation we were unable to examine the blood of all the captured animals owing to putrefaction. Our studies were carried out on forty-nine adult roussettes, four young ones with umbilical cord still present, and three foetuses. Out of the fifty-six bats examined, two only proved infected. Gametocytes were found in the blood of one of the young ones and also in the blood of one of the foetuses. This rather peculiar observation led us to undertake a painstaking examination of contact smears of the organs of the foetus, and to the discovery in the spleen of extremely rare schizogonic stages.

Of the two bats captured alive and brought back from this expedition, one was kept under observation from 18 August 1947 to 1 March 1948, when it died after more than 6 months' captivity. There were no clinical signs prior to death and no macroscopic changes at autopsy. In both the smears and the impression preparations we found trophozoites and rosettes in abundance. The second bat died after more than a year's captivity, on 28 September 1948. No plasmodia were ever found during life or after death in either blood or tissue smears.

Our second trip did not produce any material related to this problem. During our third expedition, between 16 and 18 April, we found four infected roussettes. One of them reached Europe. We were able, by repeated examinations of this bat, to study the sexual and the asexual stages of the plasmodium; but once the host became adapted to its new surroundings the parasites disappeared from the blood and could never be found again.

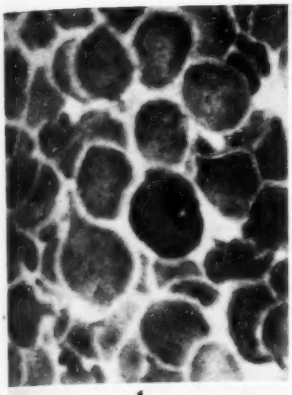
With the exception of tissue forms, we have thus observed all the developmental stages of the parasite in its vertebrate host, i.e. asexual forms and gametocytes. Several observations gave us the impression that we were dealing with a benign infection, which may become apparent after such a shock as that of capture, or intercurrent infection. Latent parasitism may very well be more frequent than our results tend to show.

V. DISCUSSION

In all the published data the most striking observation is the frequent predominance of gametocytes in the peripheral blood, and the extreme scarcity of schizonts. In *Plasmodium melanipherum* Dionisi did not observe any schizonts. Schingareff alone described and drew forms in the last stage of schizogony, some free and others included in leucocytes. In *P. epomophori*, Rodhain did not see more than a fragmentation of the nucleus of the schizont into six to ten chromatin blocks. Finally, the segmenters observed by Manwell are rather special as they do not show any pigment and look like the tissue forms of avian Plasmodia. On the contrary, the plasmodium found in *Roussettus leachi* presents, both in the blood and in the organs, typical and sometimes very numerous rosettes (Pl. IV, fig. 6).

The absence of pigmented schizonts, at least in a number of cases, forms the basis of Manwell's opinion that the plasmodia of *Chiroptera* are probably nearer to the genus *Haemoproteus* than to *Plasmodium*. He considered the possibility of creating a new genus, with intermediary characters between those of *Haemoproteus* and *Plasmodium*. This suggestion does not seem to be justified in the case of the parasite of the roussette, as we have found in this species the whole erythrocytic cycle, with all the morphological characters of a *Plasmodium*.

The congenital infection of the roussette is peculiar, especially as only gametocytes could be found in the blood of the infected foetus. Limbos (1948), in the Belgian Congo, observed rare asexual



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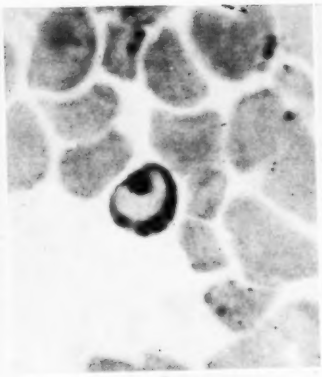

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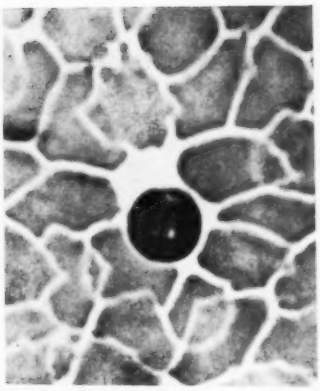

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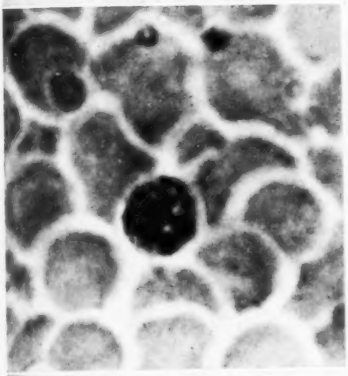

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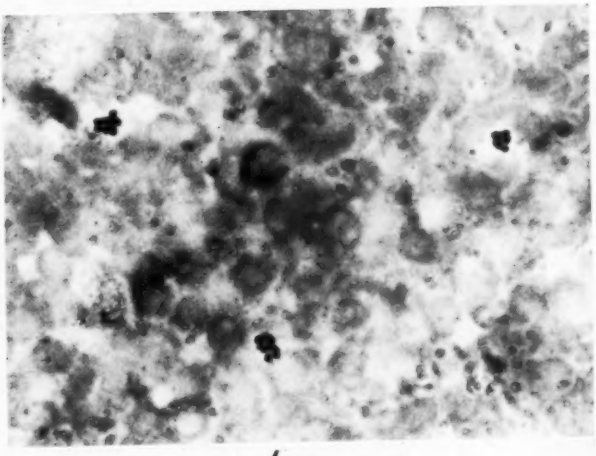

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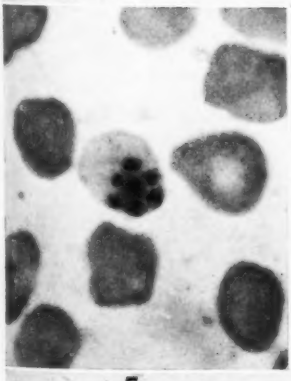

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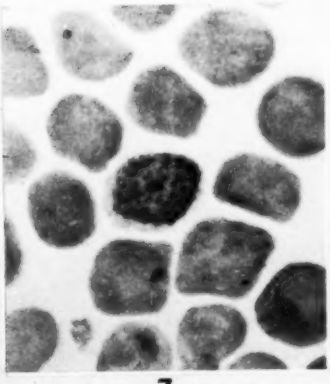

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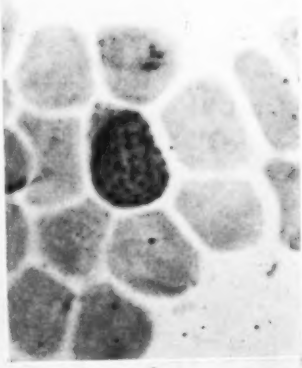

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forms and some gametocytes of *P. falciparum*, 22 hr. after birth, in the peripheral blood of the infant of a native woman suffering from malaria.

We suggest that the parasite we have just described should be called *P. roussetti*, at least temporarily. The soundness of creating, as certain authors do, new species, based solely on the morphology of the gametocytes and the character of the pigment appears doubtful, but our description of the various stages of the sexual and asexual development of the pigmented parasites, appears to us to justify the temporary creation of a separate species. Here, as in other fields of microbiology, morphological criteria are only sufficient for a temporary definition of species. Final specific identification will only be possible after inoculation of the various plasmodia into Chiroptera, or eventually into laboratory animals.

VI. SUMMARY

The intra-erythrocytic development of a parasite of the frugivorous bat, *Roussettus leachi*, has been observed. The name *Plasmodium roussetti* has been suggested temporarily. The rosettes are typical of the genus *Plasmodium*. The parasites can be transmitted congenitally and gametocytes have been found in foetal blood.

This work has been made possible by grants from the Fonds National de la Recherche Scientifique and from the Institut pour la Recherche Scientifique en Afrique Centrale.

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EXPLANATION OF PLATE IV

Fig. 1. Young trophozoite.
Fig. 2. Free large ring.
Fig. 3. Immature schizont.
Fig. 4. Schizont with four blocks of chromatine.
Fig. 5. Mature segmenter with eight merozoites.
Fig. 6. Three rosettes in one microscopic field.
Fig. 7. Macrogametocyte.
Fig. 8. Microgametocyte.

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OBSERVATIONS UPON A TRICHOMONAD (PROTOZOA: MASTIGOPHORA) FROM THE GUT OF THE SNAKE, *VIPERA AMMODYTES* L.

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(With 13 Figures in the Text)

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I. INTRODUCTION

The organism described in this paper was isolated in 1940 from the rectum of a Nose-horned Viper, *Vipera ammodytes* L., from Bulgaria by Dr Otto Jírovec, who kindly provided me with a culture of the flagellate. It was at that time supposed to be a species of *Eutrichomastix* (Jírovec, 1941–4) similar to *E. viperae* recorded by Léger in 1904 from *Vipera aspis*, the European Asp, and the following work was undertaken to determine its exact taxonomic status and provide an adequate description of this species. It was early apparent that it did not belong to the genus *Eutrichomastix*, and I have decided that it is a strain of *Trichomonas batrachorum* Perty, 1852. It is not yet possible to define with exactitude the limits of distribution of this well-known species, nor indeed of many other trichomonads named from poikilothermic vertebrates. If the taxonomy and distribution of these parasites is ever to be cleared up, it will have to be based on more exact inquiry than has usually been given in the past. So much uncertainty at present prevails in this field, that I propose to set out in detail the evidence for my conclusions in this one case.

II. METHODS

(i) *Cultivation* in vitro

Excellent cultures have been maintained for 10 years (3 years in my care) using the all-liquid medium recommended by Jírovec (1941–4), i.e. 0·6 % saline solution containing 5·0 % serum, without additional carbohydrate; horse—instead of human—serum is satisfactory. The bacterial population amply supplied the nutritional needs of the flagellates without overgrowing the Protozoa, which needed subculture only once in 3 or 4 weeks. In the richer medium (HShs) of Dobell & Laidlaw (1926) the flagellates also grew well, but bacterial growth was so rapid that subcultures had to be made every 10 days.

Provided with sterile rice starch, the trichomonads ingested the grains avidly till their outlines became irregular and their organelles obscured; but no marked increase in the numbers of individuals was evident. On subculturing such flagellates into starch-free medium, it was some weeks before the grains were finally absorbed: evidently digestion was very slow. The addition of 0·5 % dextrose did not help the protozoans, but so encouraged bacterial growth that the subcultures died out in a few days.

(ii) *Cytological technique*

As much use as possible was made of observations on the living organisms to supplement the pictures given by stained preparations, especially in the study of division.

In making permanent preparations it was difficult, even with the aid of egg albumen, albumen-glycerine or serum, to attach the flagellates so firmly to the slide that they remained there during subsequent treatment. The problem was eventually solved by making use of a celloidin method, used in these laboratories by Dr R. S. J. Hawes. As this technique overcomes a very common difficulty, I offer particulars of it here.

A culture of the organisms is poured into a 15 c.c. conical centrifuge tube and spun for about a minute (a hand centrifuge is the most useful), the supernatant fluid is carefully removed with a pipette, and the deposit washed once or twice with 0·6 % saline solution; without this washing, the medium will precipitate on the addition of fixatives. After the final spinning and removal of excess fluid, about 10 c.c. of fixative are poured on to the deposit, which

should have been concentrated to about 0·2 c.c., and the whole is well mixed with a pipette. The post-fixation treatment of the material is also carried out 'in bulk' in the centrifuge tube. After thoroughly washing out the fixative and dehydrating the material (two changes of absolute alcohol are advisable), about 0·2 c.c. of a thin celloidin solution are dropped on to the final dehydrated deposit. 10 mg. of Gurr's powdered celloidin in 10 c.c. of equal parts absolute alcohol and ether gives a suitable concentration, and when run through a Whatman's no. 2 filter-paper, produces a rapid succession of drops. There is now a concentrated suspension of fixed, dehydrated flagellates in a very small volume of celloidin at the bottom of the tube. A little of this suspension is withdrawn by a fine pipette fitted with a firm rubber teat which can be regulated easily. One drop placed on a clean glass slide should spread over a circular area 2 cm. in diameter. The solvent evaporates quickly, and when the patch of celloidin is almost dry, the slide is dipped in 96 % and may be stored in 70 % alcohol. The Protozoa are now well distributed and firmly fixed to the slide. The viscosity of the celloidin solution is important—if too thick, the drop will not spread evenly on the slide and the scab of celloidin containing the organisms may become detached on subsequent passage through staining solutions. If too thin the material will spread over too large an area and the amount of celloidin will not be sufficient to stick the flagellates on firmly. It is easy to regulate the viscosity after a single trial, by adding a little absolute alcohol and ether to thick solutions, or allowing thin ones to run two or three times through a filter. The technique is not difficult to acquire and will amply repay practice.

The most useful fixatives were Flemming's (strong formula) and Champy's fluids. Both caused practically no distortion of the organism; the former gave good nuclear pictures and the latter revealed details of the organelles extremely well. Bouin's fluid, and Schaudinn's fluid, with or without acetic acid, also proved useful, but some distortion occurred, and the cytoplasm had a much coarser appearance.

For general purposes, Heidenhain's iron haematoxylin, used after the organisms had been hardened in 96 % alcohol for 48 hr., mordanted for 24 hr. and stained for the same length of time, gave good results. I was unable to demonstrate mitochondria by Janus Green, Benda's or the Champy-Kull method. Rafalko's (1946) modification of Feulgen's technique for chromatin, and Sudan IV in alcoholic solution for fat, were also used.

III. STRUCTURE OF THE TRICHOMONAD

The trophic individual (Fig. 1) is fusiform in shape, and the average size of living organisms without the projecting tips of the axostyles is $16 \times 8\ \mu$. There is some longitudinal shrinkage on fixation to $13 \times 8\ \mu$. 'Giant' forms (Fig. 13) as large as $22 \times 21\ \mu$, subspherical and less active, were always present in my cultures and became more numerous with increasing age. As their cytoplasm was generally crowded with deeply staining parasites, the organelles were somewhat obscured; as far as could be seen, they were quite normal. A few double individuals and quadrinucleate 'giants' with four corresponding sets of organelles occurred in cultures of all ages as recorded for *T. hominis* by Bishop (1931).

The *cytoplasm* is finely granular and may be vacuolated, some vacuoles containing bacteria. The larger cytoplasmic granules appear as greenish refractory bodies in the living organism, and acquire a reddish tint when the flagellates are exposed to 1:10,000 Neutral Red for 1 hr. or more. Sudan IV gave no fat reaction in this strain, but was used successfully on *T. vaginalis* as a control. Hyperparasites of the *Sphaerita* type are commonly present in the older individuals of this strain, and are especially numerous in the 'giants'.

The oval *nucleus*, $3{\cdot}5 \times 3{\cdot}0\ \mu$, lying near the anterior end of the body, can be seen in the living organism as a homogeneous, greenish structure of greater density than the surrounding cytoplasm (Fig. 1). The trophic nucleus (Figs. 2–5) has a clearly defined membrane and a prominent central or slightly excentric karyosome, staining deeply and surrounded by a narrow clear zone, the halo. In material fixed with Flemming's solution the karyosome is regular in shape and stains uniformly, but after fixation with Bouin's or Schaudinn's solution it appears swollen, often irregular in outline, and less strongly siderophilic in the centre. Nuclei with two karyosomes were sometimes encountered. The region between the halo and the nuclear membrane appears to consist of finely granular material, more densely staining than the cytoplasm but devoid of discrete chromatin particles. No rhizoplastic connexion could be demonstrated between the nucleus and the blepharoplast.

The anterior flagella, parabasal apparatus, costa, and axoneme of the undulating membrane arise from a *blepharoplast* in front of the nucleus (Fig. 4). The head of the axostyle abuts on to the blepharoplast, and the anterior ends of the cytostome lips lie very close to it. The blepharoplast is not visible in living individuals and does not stain well in preparations fixed with Flemming's solution; after fixation with Bouin's or Champy's fluid, followed by iron haematoxylin, it appears to be a compact but irregularly shaped body. Its outline suggests that it is composed of a small number of granules, but I have never observed their dissociation, even at division.

The three *anterior flagella* are unequal in length, the average measurements in living organisms being

14, 17 and 22 μ. They move with extreme rapidity, lashing backwards and forwards or describing a circular course.

The deep *undulating membrane*, lying dorsally, is thrown into four or five curling folds. Iron haematoxylin stains only a single marginal filament, the axoneme, which leaves the body just short of the posterior end and is continued as a short free flagellum.

Originating from the blepharoplast and running close to the base of the undulating membrane, is the *costa*, a thin chromatic fibril. Siderophile granules, generally in one or two longitudinal rows, accompany it along the whole of its course.

The *axostyle* is a stout hyaline rod, similar in structure to that of *Trichomonas hominis* and *T. batrachorum* (Bishop, 1931); it is stained only weakly (along its edges) or not at all by haematoxylin. It is straight or slightly bent, and the swollen anterior end abuts on the blepharoplast and curves ventrally round the nucleus to which it is closely applied. Posteriorly it projects beyond the body for about one-third of its length and tapers to a fine point, the needle-like process contrasting markedly with the blunt terminations of the axostyles of *T. augusta* (Kofoid & Swezy, 1915) and *T. muris* (Wenrich, 1921). No chromatic rings are present at its point of emergence.

The *parabasal apparatus* lies at the anterior end of the body on the dorsal side, close to and usually slightly to the right of the costa. In trophic individuals it consists of two rather short, generally parallel, rods each attached to the blepharoplast by a short thread. In material fixed with Champy's fluid and stained with iron haematoxylin the two rods appear as homogeneous, densely staining bodies (Fig. 3), but after Flemming's fixative with 5·0% acetic, each is seen to comprise a siderophile fibre invested with a thick sheath of less deeply staining material (Figs. 4, 5), closely resembling the parabasal of *Ditrichomonas termitis* (Cutler, 1919). Bouin's fluid renders the structure unrecognizable, and after Schaudinn's fixative it does not stain at all. The constancy with which these two rods occur is remarkable—in individuals with apparently only one, a second can generally be discerned below it by careful focusing. This form of parabasal is also present in a strain of *Trichomonas batrachorum* from the Fire-bellied Toad, *Bombinator igneus*, and which in all other details agrees with the existing descriptions of that species (Grassé, 1926; Bishop, 1931).

The *cytostome* (Figs. 1–6) is clearly visible in trichomonads fixed with either Flemming's or Champy's fluids as a long, narrow cleft at the anterior end of the organism on its ventral surface, reaching back almost to the posterior border of the nucleus. The deeply staining edges of the two lips may be slightly wavy.

IV. THE DIVISION PROCESS

At the onset of mitosis, the finely granular material within the nuclear membrane gradually disappears, and is replaced by a small number of discrete particles, eventually recognizable as the chromosomes. Their affinity for stains, at first weak,

Legends to Figs. 1–13

Figs. 1–13. *Trichomonas* from *Vipera ammodytes*. The figures were drawn with the aid of a Zeiss camera lucida, under a Watson 2 mm. apochromatic oil immersion lens (N.A. = 1·37) and are × 1875. All preparations were stained with Heidenhain's iron haematoxylin. In most cases, the anterior flagella have been cut off to facilitate arrangement on the page. Abbreviations: Ch., Champy's fixative. Fl., Flemming's fixative with 5·0 % acetic acid.

Fig. 1. Trophic individual drawn from living material.

Fig. 2. Trophic individual—parabasal apparatus not visible. Fl.

Fig. 3. Trophic individual showing parabasal. Ch.

Figs. 4, 5. Trophic individuals. Fl.

Fig. 6. Individual with early prophase nucleus. Fl.

Fig. 7. Slightly later stage than Fig. 6. Prophase granules in the nucleus more distinct, karyosome irregular, nuclear membrane thinner, organelles less deeply stained. Fl.

Fig. 8. Metaphase trichomonad showing centrodesmus with one parabasal rod at each end. Chromosomes not very clear; tip of old axostyle still visible. Fl.

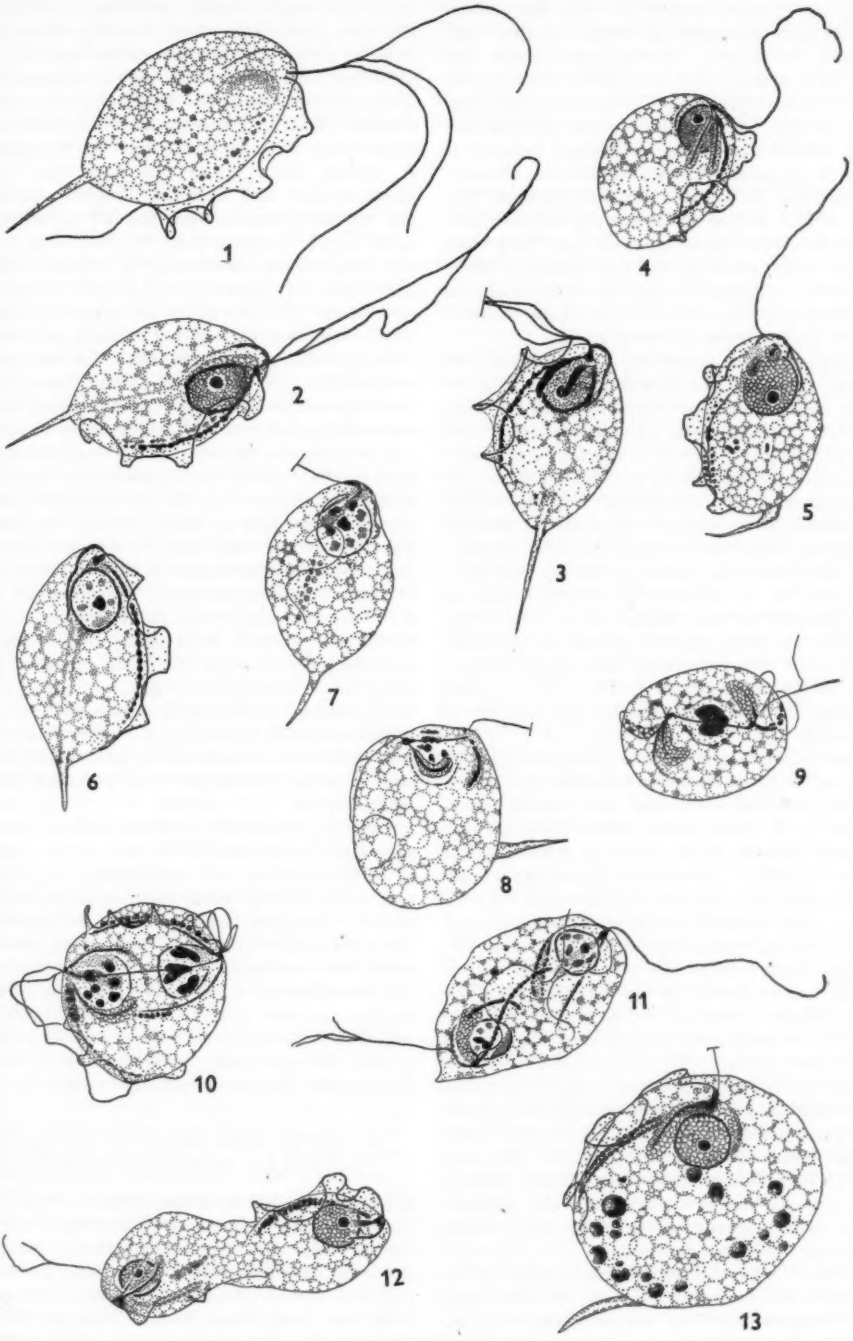
Fig. 9. Late metaphase. One parabasal element, costa, and undulating membrane at each end of the centrodesmus. Chromosomes lying close together in the equatorial region. Fl.

Fig. 10. Late anaphase. Chromosomes being drawn polewards as the centrodesmus lengthens. Two costae, undulating membranes and sets of anterior flagella present; new axostyles growing out from the blepharoplasts. New membranes are forming round the daughter nuclei, the one on the left being at a more advanced stage. Fl.

Fig. 11. Telophase. Reconstitution of the daughter nuclei is proceeding. Fl.

Fig. 12. Later telophase individual with the daughter nuclei in the trophic state, and each parabasal apparatus consisting of two rods. The cytoplasm is about to divide; only part of one of the new axostyles is visible. Fl.

Fig. 13. 'Giant' form with parasites in the cytoplasm. Fl.



Figs. 1–13.

gradually increases; meanwhile the karyosome swells, becomes irregular in outline, stains only feebly and eventually disintegrates before the chromosomes attain their maximum density. In this respect it behaves, as in *Trichomonas vaginalis*, like a nucleolus. A typical prophase nucleus has a clearly defined membrane enclosing a number of spherical or irregularly shaped bodies, the chromosomes, disposed throughout a colourless area. The trophic nucleus is Feulgen-negative, but the prophase chromosomes give a faint reaction. Their close proximity to one another, and the obscuring effect of organelles overlying the nucleus, make it impossible to determine the exact number of the chromosomes, but there appear to be about six.

The nucleus now increases in size, and the membrane becomes less clearly defined (Fig. 8). The two centrosomes—presumably formed from the dividing blepharoplast, though it has not been possible to verify this—come to lie at opposite poles of the nucleus, connected by a thread-like, deeply staining centrodesmus. The cluster of chromosomes, now elongated, lies equatorially (Fig. 9), but without exhibiting anything like the 'diagrammatic' arrangement on an equatorial plate figured by Kofoid & Swezy (1915) for *T. augusta*. The chromosomes lie roughly parallel to the centrodesmus. Prophase stages were common, and the process is evidently prolonged; but metaphase must take place rapidly, for it is rarely encountered in films and in none was it possible to count exactly the number of chromosomes.

The chromosomes divide; their orientation, in the long axis of the nucleus, before and after division, is consistent with the view that they divide transversely as in *T. batrachorum* (Bishop, 1931) and *T. vaginalis* (Hawes, 1947). (See Fig. 9 and Fig. 10, right-hand nucleus.) No spindle fibres are visible now or at anaphase, but the chromosomes lie with their long axes parallel to the centrodesmus and appear to converge upon the centrosomes (Fig. 10). Membranes form around the two daughter nuclei, the chromosomes break up into granules and the typical trophic nucleus is reconstituted (Fig. 11) before cytoplasmic division begins. The two groups of daughter chromosomes often do not travel simultaneously towards the poles, one set lagging behind so that one daughter nucleus is formed before the other.

During mitosis, the organelles lose much of their affinity for stains (Bishop, 1931; Hawes, 1947) and this makes their fate at division extremely difficult to determine. The following is a composite account based on details gleaned from many individuals, each defective in some particulars. The blepharoplast divides into two during prophase, either before or just after the appearance of the chromosomes, and the complement of six flagella is made up by growth from the blepharoplasts: they reach their maximum length during prophase. At metaphase, the two centrosomes can be seen close to, but distinct from, the blepharoplasts and remain so until early telophase. The deeply staining centrodesmus persists until the daughter nuclei are reconstituted; it does not contribute to the new organelles which may be complete before its disappearance. A second costa grows out from the daughter blepharoplast and in most anaphase individuals the two fibres can be seen, the old one thicker and more deeply staining than the new (Fig. 10). The new undulating membrane is formed relatively early and very quickly and is fully developed at metaphase. The fate of the axostyle at division has been dealt with at length by many authors. (See Bishop (1931) and Hawes (1947) for discussions on this subject.) In the trichomonad here described, the old axostyle disappears during metaphase and is replaced by two new ones which, early in telophase, can be seen growing out from the daughter blepharoplasts as thin fibres curving round the nuclei on the side opposite the costa. The newly formed axostyles vary considerably in their affinity for stains; in Fig. 12 only a trace of one of them can be seen, but in the living trichomonads at the telophase, the tips of both daughter axostyles are generally visible. The two rods comprising the parabasal apparatus become separated from each other when the blepharoplast divides, one being attached to each daughter blepharoplast (Figs. 8, 9). At telophase both daughter parabasals consist of the two separate rods characteristic of the trophic individual (Fig. 12). The cytostome disappears at metaphase, and two new ones are evidently formed during telophase (Figs. 11, 12).

During division, the trichomonads are extremely active and swim rapidly about. In the cytoplasm of individuals at the telophase, the organelles constantly change their relative positions: at one time the daughter nuclei will lie close together, with the axostyles, costae and undulating membranes parallel to each other, while a moment later one set has slewed round through 180° carrying its nucleus to the opposite pole of the body. Each set of organelles can turn round independently on its axis, so that the undulating membranes may lie on the same or on different sides of the organism.

V. INFECTION OF *BUFO VULGARIS* TADPOLES WITH TRICHOMONADS

On morphological evidence alone it would be rash to separate the trichomonad from *Vipera ammodytes* from *Trichomonas batrachorum* Perty, 1852, which it closely resembles, but as I am only aware of one previous record of this species from an aphidian host—the Long-nosed Snake (Trussell, 1947), and Bishop (1934) could not infect *Bufo vulgaris* or

Rana temporaria tadpoles with a strain of *Trichomonas lacertae* from the Green Lizard, *Lacerta viridis*—I have thought it necessary to seek further evidence by experiments. An attempt was therefore made to infect trichomonad-free toad tadpoles with (*a*) *Trichomonas batrachorum* and (*b*) the snake trichomonad.

(i) *Experimental method*

Spawn from *Bufo vulgaris*, a known host of *Trichomonas batrachorum* (Alexeieff, 1909; Dobell, 1909; Bishop, 1931, 1934) was collected from a pond and brought to the laboratory where it was washed thoroughly with tap water and distributed among four alcohol-cleaned tanks of tap water covered with muslin to exclude dust. When the eggs hatched, shreds of sterile Marmite agar, and watercress, previously treated with 10% alcohol for 15 min., were added to the tanks. The young tadpoles ate this diet readily, and bulk samples of their faeces from each tank were examined microscopically and inoculated into tubes of serum-saline medium. Subsequent examination of the faeces of infected tadpoles showed that, although trichomonads cannot always be seen on direct microscopic examination, cultures made from such samples are rich in the parasites.

Each tank was examined in this way six times during the succeeding 5 weeks. A variety of freshwater and coprozoic Protozoa were found in the faecal pellets on direct microscopic examination, and these sometimes persisted in culture. Each culture tube was looked at three times during the month following, and all were negative for *Trichomonas* except the fifth sample taken from tank 2 which showed numerous trichomonads on the second examination. This tank was discarded. Samples of tap water, and the watercress used for feeding, were also cultured and proved negative for *Trichomonas*. Three days before infecting the tadpoles, the faeces of each one were examined separately: again no trichomonads were found. The stools of these tadpoles had then been examined seven times in all, six in bulk and once individually.

Three days after the last faecal examination, the tadpoles were distributed among three clean tanks of tap water, twelve in each. To one tank were added cultures of *T. batrachorum* and to another, cultures of the trichomonad from *Vipera ammodytes*, while the third was left as a control. I had previously confirmed Bishop's (1934) statement that individuals of *Trichomonas batrachorum* survive for some time at the bottom of 9·0 cm. columns of water, but die after a few hours in shallow dishes. (I used tap instead of filtered rain water.) Three-week-old cultures, some grown in ordinary serum-saline medium and some in this medium diluted two-thirds its strength with tap water, were used for the feeding experiments. Centrifuged deposits of the cultures were washed and pipetted on to the agar and watercress leaves which the tadpoles were eating. The tanks were inoculated in this way on two successive days. Samples of the inocula gave excellent cultures of *Trichomonas* in serum-saline medium.

(ii) *Results*

After the tadpoles had been fed with the parasites, the faeces of each were examined twice. On the first occasion, 3 days later, eleven of the tadpoles fed with *T. batrachorum* and only three of the group fed with the snake *Trichomonas* gave positive cultures of the flagellates. Direct microscopic examination of the faeces showed, in some cases, spherical bodies which may have been rounded-up and immotile trichomonads, although not all faecal samples containing them gave rise to positive cultures (see Table 1). All the control tadpoles were microscopically and culturally negative. Three weeks later, positive cultures were obtained from all the experimental tadpoles, but from none of the controls, one of which had died. Microscopic examination of the excreta showed living trichomonads in samples from one tadpole infected with *T. batrachorum* and one infected with the snake *Trichomonas*; the spherical bodies mentioned above were present in one faecal sample from the latter group only. On the same day, the tadpoles were killed, their guts removed aseptically and teased out in sterile 0·6% saline solution, and examined. Active living trichomonads were recovered from all the experimentally infected tadpoles, both on direct microscopic examination and in culture, while all eleven controls were negative. Careful study of both fresh and fixed stained preparations showed that the trichomonads cultured from the faeces and intestines were morphologically identical with the original strains of *T. batrachorum* and the snake *Trichomonas*.

The results are summarized in Table 1.

These experiments show that the demonstration of trichomonads in tadpole faeces by cultural methods is a reliable indication of their presence in the intestine: positive cultures from faeces were obtained from all the infected tadpoles on the day they were killed. Failure to culture the parasites from the excreta of a number of the tadpoles 3 days after the last feed with trichomonads suggests either that no infection had taken place at that early date, or only a very slight one in which few flagellates were being passed in the faeces at irregular and long intervals.

VI. DISCUSSION

The parabasal. In the snake *Trichomonas* described above, and in the strain of *T. batrachorum* from *Bombinator igneus*, the parabasal apparatus comprises two parallel rods, a condition not previously

recorded for any trichomonad.[1] Owing to the difficulty of staining it satisfactorily, the parabasal of *Trichomonas batrachorum* has not received as detailed a study as the other organelles, and its structure is not known with certainty. Bishop (1931) refers to it as a bag-like structure hanging from the mophile and chromophobe parts, lying ventral to the nucleus and only seen in specially prepared slides. A more exact account of the parabasal in trichomonads from amphibian hosts is necessary for a profitable discussion of its structure.

Conclusion. Trichomonad parasites from ophidian hosts have been recorded a number of times (Dobell, 1910; Plimmer, 1912; Wenyon, 1926; Reichenow, 1929; Das Gupta, 1927, 1935, 1936*a*, *b*), but the trichomonad under discussion bears less resemblance to any of them than it does to *T. batrachorum*. In morphology, method of division, and cultural requirements the two are identical, and both will infect toad tadpoles readily. It has not been

Table 1. *The results of feeding* Bufo vulgaris *tadpoles with trichomonads*

	Examination of faeces before feeding		Examination of faeces after feeding				Examination of intestines after feeding	
			3 days		24 days			
	Micr. exam.	Culture	Micr. exam.	Culture	Micr. exam.	Culture	Micr. exam.	Culture
Twelve tadpoles infected with *Trichomonas batrachorum*	−	−	⊙	+	−	+	+	+
	−	−	−	+	−	+	+	+
	−	−	⊙	+	−	+	+	+
	−	−	−	+	−	+	+	+
	−	−	⊙	+	−	+	+	+
	−	−	⊙	+	+	+	+	+
	−	−	⊙	−	−	+	+	+
	−	−	⊙	+	−	+	+	+
	−	−	⊙	+	−	+	+	+
	−	−	⊙	+	−	+	+	+
	−	−	−	+	−	+	+	+
Twelve tadpoles infected with *Trichomonas* from *Vipera ammodytes*	−	−	−	−	+	+	+	+
	−	−	−	−	−	+	+	+
	−	−	−	−	−	+	+	+
	−	−	⊙	−	−	+	+	+
	−	−	−	−	−	+	+	+
	−	−	⊙	+	⊙	+	+	+
	−	−	⊙	−	−	+	+	+
	−	−	−	+	−	+	+	+
	−	−	−	−	−	+	+	+
	−	−	−	+	−	+	+	+
	−	−	−	−	−	+	+	+
	−	−	−	−	−	+	+	+
Twelve controls	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−
	−	−	−	−		(One tadpole died)		

\+ Denotes active trichomonads present.
⊙ Denotes spherical bodies, possibly rounded-up trichomonads.
− Denotes absence of trichomonads.

[1] Since this paper was submitted for publication, records of a 'double' parabasal in *Trichomonas natricis* from *Natrix natrix* and *Natrix viperinus* (Coutelen *et al.*, 1948) and in *Tritrichomonas* (= *Trichomonas*) *batrachorum* (Honigberg, 1950) have been found.

possible to effect the converse experiment and infect *Vipera ammodytes* with *Trichomonas batrachorum*, but this does not seriously affect the conclusions I have drawn, namely, that the trichomonad from *Vipera ammodytes* is not a separate species but merely a strain of *Trichomonas batrachorum* which had established an infection in the gut of that particular snake.

VII. SUMMARY

1. The structure, method of division and cultural requirements of a trichomonad from the gut of *Vipera ammodytes* L. have been studied; the organism was found, in these respects, to be identical with *Trichomonas batrachorum* Perty, 1852.

2. Tadpoles of *Bufo vulgaris* have been infected experimentally with the strain.

3. It is concluded that the trichomonad was a strain of *Trichomonas batrachorum* established in a reptilian host.

I wish to thank Prof. Dr Otto Jírovec of the Universita Karlova, Prague, for the cultures of *Trichomonas batrachorum* and the trichomonad from *Vipera ammodytes*; Dr E. G. Pringsheim of the Botany School, Cambridge, for putting me in touch with Dr Jírovec; the Medical Research Council for a grant which has made this work possible; and the Department of Zoology of this University College for providing the research facilities. To Dr R. S. J. Hawes, my Supervisor, I would like to express my gratitude for his ready help and invaluable encouragement and advice.

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OCCURRENCE OF *GLOBIDIUM GILRUTHI*, A PROTOZOON PARASITE OF SHEEP AND GOATS, FROM THE INDO-PAKISTAN SUB-CONTINENT.

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During the collection of helminths from a goat, millet-sized nodules were noticed in the mucous membrane of the abomasum. Examination of these nodules showed that there were no larval nematodes in their substance, but that they contained a large mass of spores. The nodules were identified as cysts belonging to the protozoon parasite, *Globidium gilruthi*. This parasite has not previously been recorded from the Indo-Pakistan sub-continent. The parasite was for the first time observed by Flesch (1883). Moussu & Marotel (1902) regarded it as a developmental stage of the coccidium, *Eimeria faurei*. It was studied by Gilruth (1910) and in the same year by Chatton (1910), who named it as *Gastrocystis gilruthi*.

Examination of fifty abomasa of sheep and goats at the Lahore slaughterhouse, where animals are derived from different parts of Pakistan, showed that seventeen of them were infested with this parasite. Among 180 animals examined in the Dera Ghazi Khan, Muzaffargarh, Mianwali and Sargodha district 169 were found to be infested. It appears that *G. gilruthi* is a common parasite of sheep and goats in the Punjab. Triffitt (1925) found *G. gilruthi* in as many as 92 % of British sheep.

The cysts have a diameter of 200–500 μ and are situated within little opalescent elevations of the mucosa mainly in the cardiac portion of the abomasum. Each cyst is enclosed by a definite wall which has concentric striations. The colour of the immature cysts is opalescent while the mature cysts are distinctly white. At one place in the wall there is a large flattened nucleus which may be 80 μ long by 10 μ broad. Within the mature cyst is a mass of spores, each of which measures 10 by 1·5 μ. One end of the spore is blunt and the other pointed, the nucleus being situated near the blunt end, while the other end is clear and hyaline.

In the majority of the animals the infection is light, but it may sometimes be heavy. Because the cysts, when they are mature, rupture into the stomach, haemorrhages may be caused when infections are heavy and serious symptoms may follow.

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NOTES ON THE MORPHOLOGY AND LIFE HISTORY OF *SCHISTOCEPHALUS SOLIDUS* (CESTODA: DIPHYLLOBOTHRIIDAE)

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(With Plate V and 17 Figures in the Text)

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I. INTRODUCTION

Schistocephalus solidus is a small avian pseudophyllidean cestode which, on account of its small size and the ease with which its plerocercoid may be cultured to maturity *in vitro*, has been used within recent years for the study of cestode physiology.

In several aspects of this work it is necesasry to have data on the life cycle, morphology and histology of the adult and larvae. Much of this information is scattered throughout the literature in journals not readily available; and it is the object of this paper to collect together, briefly, the salient information, supplemented with additional personal observations on large numbers of adults and larvae examined during the past 4 years.

II. HISTORICAL

S. solidus (O. F. Müller, 1776) is the only species of the genus *Schistocephalus* Creplin, 1829, of the subfamily Ligulinae Monticelli & Crety, 1891, of the family Diphyllobothriidae Lühe, 1910. The adult occurs in the intestine of fish-eating birds, the plerocercoid larva in the body cavity of fish or, occasionally, in other aquatic vertebrates. These two stages were long-considered separate species, until Bloch (1782) recognized that they were one and the same. Abildgaard (1790) confirmed this by feeding infected sticklebacks to geese, thereby obtaining an adult cestode experimentally for the first time. For some years confusion was introduced into the literature by Donnadieu (1877), who also carried out a number of feeding experiments but did not differentiate between *Schistocephalus* and the closely allied species *Ligula intestinalis*; his work was accepted for some years before the inaccuracy was discovered.

The most important contributions to the morphology of the adult and plerocercoid were made by Moniez (1881), Kiessling (1882), Fuhrmann (1896), Solowiow (1911) and Cooper (1918). The complete life cycle remained unknown until 1919 when Nybelin, experimentally, infected *Cyclops* spp. with the coracidia.

III. ADULT

(i) *Occurrence*

The adult cestode occurs in the intestine of fish-eating birds. Cooper (1918) lists as many as forty different species in which this worm has been recorded. In spite of the wide range of bird hosts, the adult is comparatively rare, a fact emphasized by several writers. Schauinsland (1885) never succeeded in finding mature *Schistocephalus* in water birds, although over a hundred birds were examined. Cooper (1918) writes: 'It is a very surprising fact that apart from Linton's report of the larva from Montana, evidently no one has up to the present found the form in any of the numerous fish-eating birds of the continent'.

This apparent scarcity of the adult in fish-eating birds was readily understandable once the life cycle of the worm became known. Schauinsland (1885) fed fish infected with plercercoids to ducks, and showed that adult worms may be passed out in the

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faeces in as soon as 36 hr. This, and later work (Smyth, 1946, 1950; Hopkins, 1950), has confirmed the fact that maturation of the plerocercoid larva is very rapid (36 hr.), and the adult worm seldom stays in the gut longer than 3–4 days. It is evident, therefore, that adults will only be found in the intestine of birds if autopsies are carried out within 3–4 days after ingestion of infected fish. Such a condition considerably reduces the chances of the adult occurring in any random post-mortem examination of birds. The number of adults found in a bird intestine will clearly depend on the fish-eating capacity of the bird in question and the percentage infection of the fish available. Abnormally heavy infections are probably fatal to the bird: Callot & Desportes (1934) found a dead duck (*Anas boschos*) with 340 adult *Schistocephalus* in the intestine, the weight of the worms being 25 g. and the weight of the bird being only 330 g.

(ii) *Size*

Lühe (1910) gives the size range: length 30–300 mm.; width 3–9 mm. These figures are quoted by subsequent authorities—Cooper (1918), Joyeux & Baer (1936). We question whether adults ever reach 300 mm. in length. The length of the adult is clearly correlated to the size of the plerocercoid for, due to the rapid maturation and short adult life, little, if any, body growth would occur in the alimentary canal, although considerable elongation due to relaxation of the longitudinal musculature would take place. The maximum size of the adult must, therefore, bear some relationship to the maximum size of the plerocercoid. Joyeux & Baer (1936) give the maximum size of the plerocercoid as 40 mm.; in our examination of many thousands of larvae, we have never found specimens longer than 50 mm. Allowing for lengthening, due to musculature relaxation (estimated at about 2½ times), it seems probable that the maximum size of the adult would not exceed *about* 125 mm. Lühe's figure of 300 mm. may be due to confusion of *Schistocephalus* with *Ligula*.

(iii) *Morphology*

Throughout the description given below, sizes of the various genitalia have been omitted as, due to the great powers of relaxation and contraction of this worm, the sizes of the organs—as seen in sections—depend to a considerable extent on the condition in which the specimen was fixed. The discrepancies in the sizes of the various organs, as measured by previous workers, have been emphasized by Cooper (1918) and we believe are due, in the main, to this factor.

(*a*) *External features.* The scolex is not marked off sharply from the head but merges into the first segment, the whole being triangular in shape (Text-fig. 17). There are no lateral bothria, but short median shallow grooves united at the top occur on the scolex. The whole strobila is ovatelanceolate with the segmentation of the proglottid overlapping subsequent proglottids, being particularly extended at the margins so that the edges appeared much serrated.

(*b*) *Musculature.* Described in detail by Kiessling (1882) and Fuhrmann (1896). There are three transverse muscle layers extending across the proglottids, being only interrupted in the immediate region of the genitalia on the aperture side. The outer and middle layers (*OTM*, *MTM*, Pl. V, fig. 2), which lie external to the vitelline glands are separated by a narrow outer longitudinal muscle band. This latter band is often difficult to locate in greatly relaxed specimens, so that in such worms the distinction between the outer and middle transverse muscles may not be clear.

Interior to the vitelline glands lie the thick inner longitudinal muscle band (*ILM*, Pl. V, fig. 2); the fibres of this layer are typically gathered into bundles which, again, are not clearly visible under low-power observation (Text-fig. 2). Internal to this layer lies the inner transverse muscle layer (*ITM*, Pl. V, fig. 2). Numerous dorso-ventral muscle fibres extend across the medulla and parenchyma; these fibres have their origin in the cells of the subcuticular region. The various muscle layers are much more clearly seen in the plerocercoid (Text-figs. 3, 4).

(*c*) *Excretory system.* Described by Fuhrmann (1896), Solowiow (1911) and Cooper (1918) in detail. There are about twenty-four to thirty excretory vessels in the medullary parenchyma with foramina secundaria piercing the cuticle at intervals.

(*d*) *Reproductive system.* The earliest trace of the genitalia appear in the 13th to the 15th proglottid, depending on the size of the strobila. The most anterior genitalia have never been found to contain

Legends to Text-figs. 1–10

Text-fig. 1. Dorsal view of genitalia in whole mount preparation.

Text-fig. 2. Adult worm; transverse section.

Text-fig. 3. Plerocercoid; Transverse section across region of male genitalia.

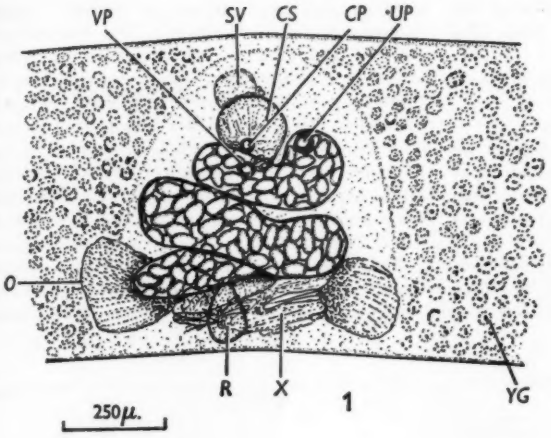
Text-fig. 4. Plerocercoid: transverse section across region of female genitalia.

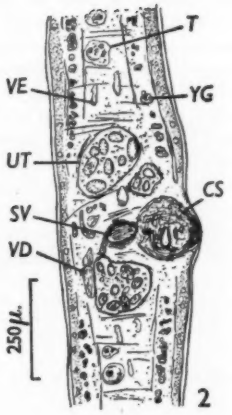
Text-fig. 5. Adult worm: sagittal section.

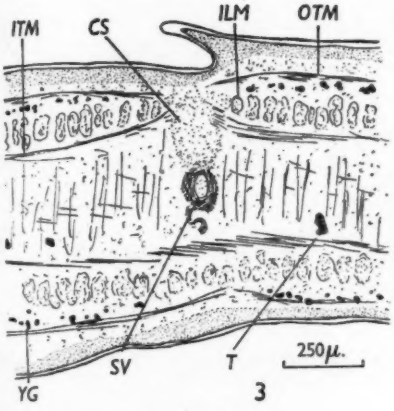
Text-fig. 6. Embryonated egg.

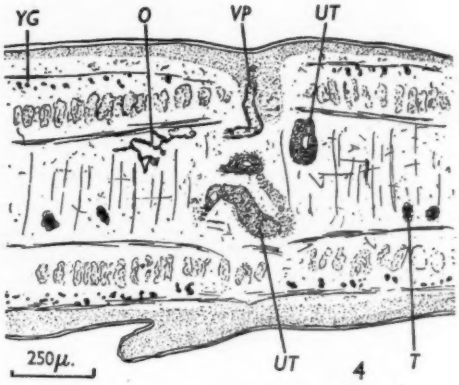
Text-figs. 7–9. Developing procercoids (after Callot & Desportes, 1934).

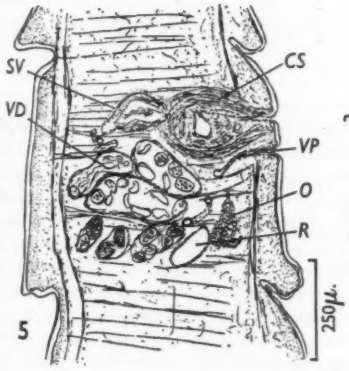
Text-fig. 10. Posterior region of *Cyclops viridis* infected with procercoids (after Callot & Desportes, 1934).











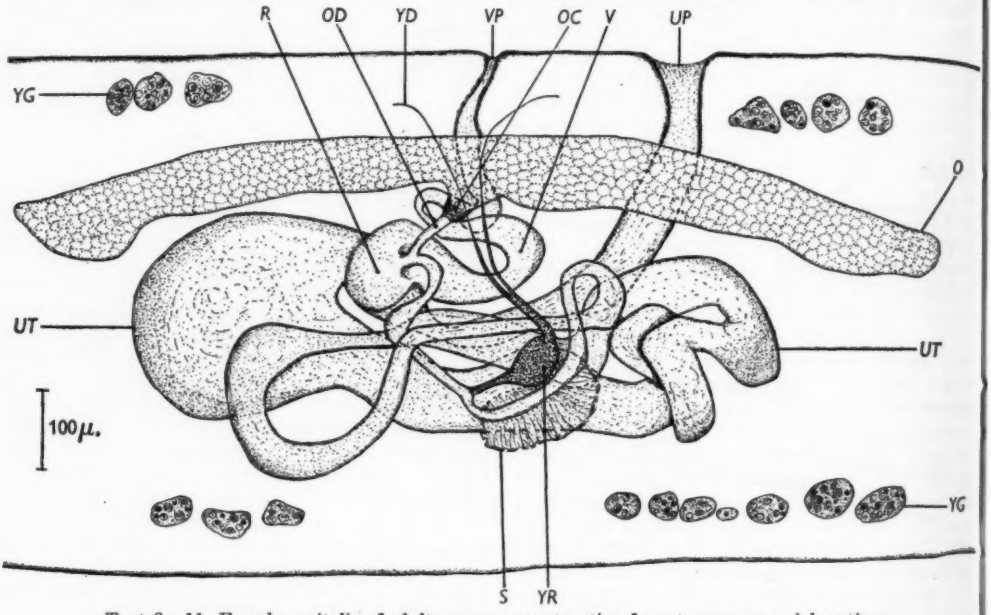
Abbreviations for all figures

CP, cirrus pore; *CS*, cirrus sac; *ILM*, inner longitudinal muscle; *ITM*, inner transverse muscle; *MTM*, middle transverse muscle; *O*, ovary; *OC*, oocapt; *OTM*, outer transverse muscle; *OD*, oviduct; *R*, receptaculum; *S*, shell gland; *SV*, seminal vesicle; *T*, testis; *UP*, uterine pore; *UT*, uterus; *V*, vagina; *VD*, vas deferens; *VE*, vas efferens; *VP*, vaginal pore; *X*, shell gland complex; *YD*, vitelline duct; *YG*, vitelline glands; *YR*, vitelline reservoir.

eggs. Eggs usually appear in the genitalia of the 15th proglottid, but occasionally in the 14th. The cirrus, vaginal and uterine openings form a triangle, the uterine pore alternating irregularly on each side of the middle line (Text-fig. 1).

The testes are arranged in a single layer in the dorsal medulla (Text-fig. 2). Spermatogenesis follows the typical cestode pattern almost identical to that described for *Ligula* (Smyth, 1949). The small size of the spermatocytes makes accurate chromosome counting difficult. From examination of large numbers of dividing spermatocytes, we conclude that the diploid number is 10–12. The vasa efferentia appear in transverse section as thin-walled vessels swollen and later shows a marked 'pulsating' movement until eventually the cirrus becomes everted with almost explosive violence. When this stage is reached, the cirrus maintains a steady in-and-out movement, a cloud of spermatozoa being ejected at the height of the evaginating movement. The eversion of the cirrus is accompanied by marked contractions of the seminal vesicle. Ejected spermatozoa show active, wriggling movements, but are very sensitive to temperature, a slight fall below 40° C. being sufficient to stop activity completely.

The vagina (*V*, Text-fig. 11), at its opening, is lined with cuticle continuous with that covering the proglottid, but soon after entering the medullary

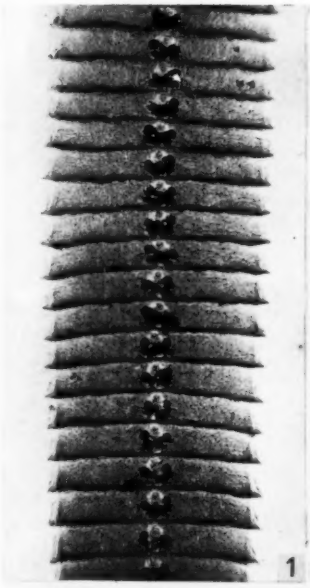


Text-fig. 11. Female genitalia of adult worm: reconstruction from transverse serial sections. Abbreviations as before.

(Text-fig. 2, *VE*) which unite to form a prominent vas deferens. The latter (Text-fig. 5, *VD*) forms a compact mass of coils, enormously distended with spermatozoa when mature, which occupies the median medulla below the seminal vesicle (*SV*, Text-figs. 1, 2 and 5).

The seminal vesicle is a thick-walled muscular structure lying slightly above the cirrus sac; it projects partly into the cirrus proper. The cirrus sac is of the typical pseudophyllidean type with numerous circular and radial muscle fibres. The cirrus occupies nearly the entire contents of the sac. The movements of the cirrus have been studied *in vitro* (Smyth, 1946, 1950). As the proglottids approach maturity, the cirrus region becomes parenchyma it becomes thin-walled, being replaced by an epithelial layer. At or near its entry into the medulla, the vagina bends sharply posteriorly; this, together with a narrowing, makes it difficult to observe in transverse sections. Caudad to the uterus, the vagina swells into a very large, thin-walled receptaculum seminis (*R*, Text-figs. 1, 5, and 11), filled with spermatozoa, lying near the posterior border of the proglottid. A short sperm duct connects the receptaculum with the oviduct.

The ovary has the typical cestode structure of the two large wings, composed of closely crowded tubules. The centre of the ovary is extended into a small isthmus leading to an oocapt at the mouth of the ovary. This oocapt is merely a slight muscular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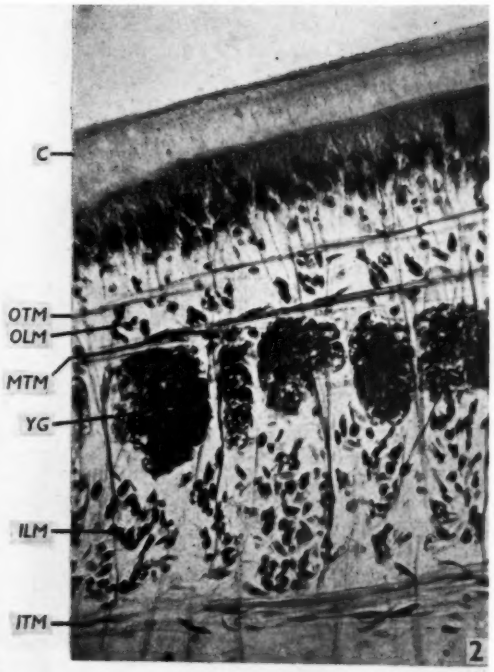
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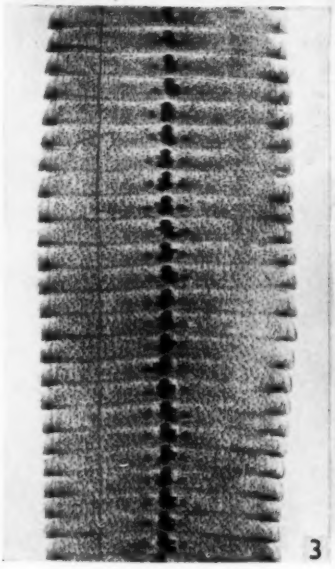
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swelling on the mouth of the oviduct; although described by Lühe (1899), it was overlooked by both Kiessling (1882) and Fuhrmann (1896). The oviduct, after receiving the sperm duct from the receptaculum, turns inwards and following a curved path passes into the end of the uterus, receiving a common vitelline duct. The latter duct is enlarged to form a yolk reservoir, which is one of the most conspicuous structures seen in sections owing to the contained mass of yolk material which stains heavily in nuclear stains. The vitelline glands are very numerous and lie between the inner longitudinal and middle transverse muscle layers. They are continuous throughout the proglottid except in the immediate region of the genitalia. Beyond the common vitelline duct the oviduct forms an ootype which is surrounded by the shell gland. As described by previous workers, the oviduct is lined with an epithelium with prominent nuclei, but no distinct cell boundaries. Beyond the ootype the oviduct passes ventrally, coils once or twice and gradually passes insensibly into the uterus. The width and extent of coiling of the uterus varies enormously with the maturity of the proglottid. When maturity is reached, the lateral coils of the uterus become so swollen that they fill the entire central region of the medulla. The shell gland, which appears in the plerocercoid as a well-defined organ, is frequently difficult to locate in the adult. Although in reconstructions (*S*, Text-fig. 11) it appears as a comparatively solid organ, in sections the shell gland cells are so loosely arranged and so enormously elongated that they may be frequently overlooked. The necks of the glands are of such a filiform nature that their actual connexions with the ootype are seldom seen.

(iv) *Eggs*

The eggs are ellipsoidal, but their size shows a great range of variation. Dimensions, according to previous workers, are: Kiessling (1882), $49 \times 34\,\mu$; Fuhrmann (1896), $70 \times 29\,\mu$; Lühe (1899), 56–38 $\times$ 38–22 μ; Linton (1927), $71 \times 43\,\mu$; Thomas (1947), $85 \times 47\,\mu$. From our material (worms matured in pigeons) we found the range to be $77–58 \times 46–35\,\mu$.

IV. LIFE CYCLE

(i) *Development of egg*

The development and hatching of the egg has been described by Schauinsland (1885), Thomas (1947) and Smyth (1950). Development follows the Diphyllobothriidae pattern, from which it does not appear to differ in any essential points. The ovum is completely enclosed by yolk follicles and is, therefore, not visible. As development proceeds, a clear area becomes apparent in the centre; this area later develops definite walls which ultimately become differentiated into a clear embryophore. In a fully embryonated egg, the embryo fills approximately three-quarters of the entire volume of the egg—only a trace of residual yolk remaining. The fully developed embryos move actively within the egg for some time before hatching as typical coracidia enclosed in a clear embryophore with long cilia. According to Thomas (1947), hatching is greatly accelerated by exposing the embryonated eggs to light, and the coracidia are capable of swimming for as long as five days.

As with most other Diphyllobothriidae, the speed of development varies enormously with temperature. According to our results, embryonation at room temperature of about 18–20° C. takes 18–20 days, but only 7–9 days at 25–26° C. The higher temperature is convenient for hatching eggs under laboratory conditions; eggs being placed in a 3 in. watch-glass in 10 c.c. of water, the latter being enclosed in a covered Petri dish to prevent evaporation.

(ii) *Development of procercoid larva*

Nybelin (1919) was the first to show that the first larval stage of *Schistocephalus* developed in copepods —experimentally infecting *Cyclops biscuspidatus* and *C. serrulatus*. The most detailed account of the procercoid development is that of Callot & Desportes (1943) who experimentally infected *C. viridis* and whose figures are reproduced here (Text-figs. 7–9). The ingested coracidia make their way through the body cavity, presumably by boring through the intestinal wall by means of their hooks. The young procercoids are round and motile but become oval and elongated, the hooks becoming constricted into a caudal appendage (Text-figs. 7–9). As the hooks are absent in the plerocercoid larva, it may be assumed that the caudal appendage and hooks are lost soon after ingestion by the fish. A fully developed procercoid (Text-fig. 9) shows a slight median depression at its anterior end and a few darkly staining clusters of cells are found in the middle region. Callot & Desportes (1934) found as many as sixty procercoids in a single copepod, filling up the entire posterior extremity of the abdomen.

There is little available evidence as to how many species of *Cyclops* can serve as hosts. In addition to the species referred to above, Thomas (1947) experimentally infected *C. leucharti*, but failed to infect other species of *Cyclops* (unnamed) or *Diaptomus*.

(iii) *Plerocercoid*

(*a*) *Occurrence*. In the British Isles we have never found plerocercoids in fish other than the three-spined stickleback *Gasterosteus aculeatus*, but according to Cooper (1918) larvae have been reported from *G. bispinosus atkinsii*, *G. cataphractus*, *Cottus poecilopus*, *C. bairdii*, *Salmo salar*, *Phoca vitulina*, *Rhynchichthys gronovii*, *Uranidea formosa*. We have examined many fresh-water lakes in England and

Ireland, and there seems no doubt that the infection is common, though local, in fresh-water areas where both *Gasterosteus* and fish-eating birds occur. One small isolated lake in Hunslet, Yorkshire, frequented by gulls, contained fish which showed a 100% infection for several years (Smyth, 1946).

A sample of twenty fish from this lake shows the extent of the infection (Table 1). It can be seen that the weight of the parasite may reach up to 92% of the actual body weight of the fish—a remarkable figure. The size and number of parasites vary greatly throughout the seasons. Very small larvae, i.e. less than 1 cm., are rarely found except in the warm summer months. An analysis of the number and size of the plerocercoids in *Gasterosteus* from the Hunslet pond over the summer months was carried out in 1946, the result of which is shown in Table 2. It was found that during May and June, in the thirty-three fish examined the greater proportion were longer than 2 cm. In August, on the other hand, over 400 larvae less than 1 cm. were obtained from thirteen fish—as many as 130 being obtained from a single fish. These figures, although based on a very limited number of observations, clearly suggest that the peak period of infection occurs during the hot summer months. This result is not unexpected, as during the winter and early spring months the low temperature of the water at the bottom of standing water would not be conducive to easy hatching of eggs. Oxygen tension and light may also be controlling factors. In our experience, fish caught during the winter and spring months usually contain one to six medium or large larvae; so that if a mass infection such as suggested in Table 2 is the normal rather than the exceptional, it would appear that there is an elimination of a large number of the initial larvae, only a few surviving.

Larvae lie free in the body cavity and we have never found evidence of encystation such as occurs with the larval stages of *Diphyllobothrium* spp. which is often found in the coelom of *Gasterosteus* in association with *Schistocephalus*.

Table 1. *Number and weight of plerocercoids of* Schistocephalus *in stickleback*

Fish no.	Weight of infected fish	Weight of fish with larvae removed	Weight of larvae	No. of larvae	Average weight of larva	% larvae / Fish
1	2·60	1·7	0·90	16	0·056	53
2	2·25	1·95	0·30	2	0·150	15
3	1·62	1·20	0·42	5	0·084	35
4	1·32	0·96	0·36	3	0·120	36
5	1·71	1·15	0·56	6	0·093	49
6	1·71	0·89	0·82	10	0·082	92
7	1·56	1·04	0·52	4	0·130	50
8	1·30	1·24	0·26	1	0·260	21
9	0·93	0·65	0·28	3	0·093	43
10	2·46	1·31	1·15	12	0·096	88
11	2·30	1·73	0·57	4	0·142	33
12	1·69	1·04	0·65	6	0·109	62
13	2·51	1·94	0·57	3	0·190	29
14	2·16	1·31	0·85	8	0·106	65
15	1·84	0·98	0·86	9	0·095	85
16	2·17	1·75	0·42	2	0·210	24
17	2·47	1·33	1·13	13	0·089	85
18	1·67	1·02	0·65	6	0·109	63
19	1·64	0·94	0·70	4	0·175	75
20	2·00	1·35	0·65	4	0·162	49

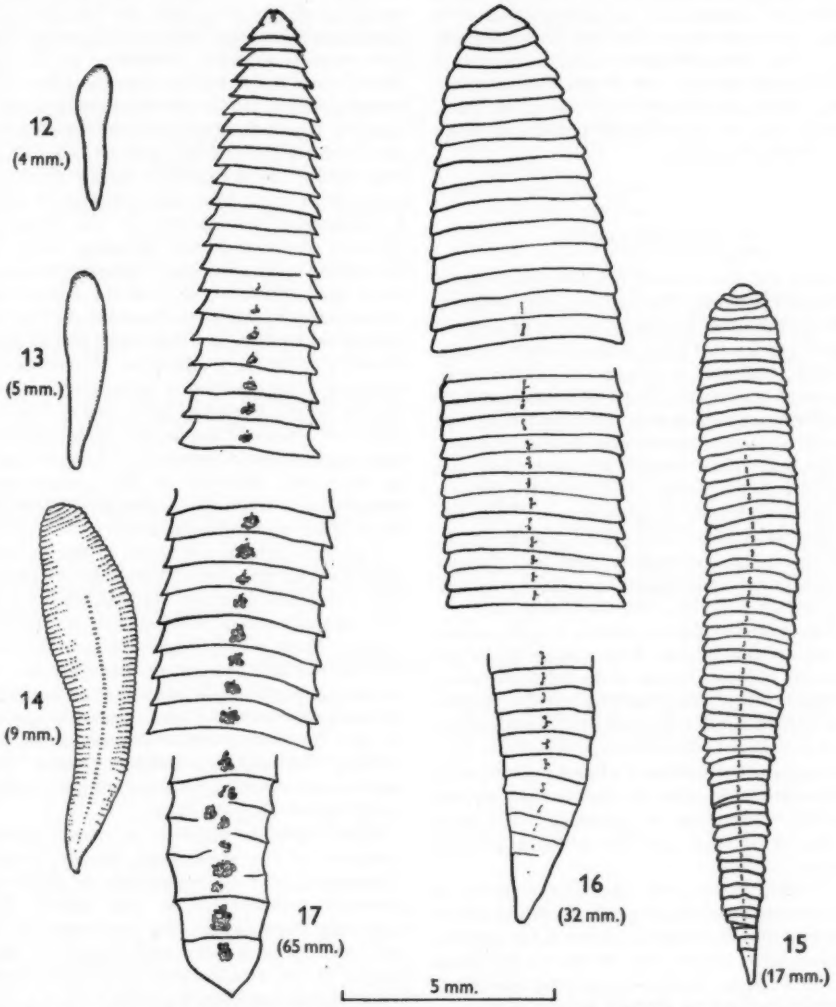
Table 2. *Analysis of size and number of plerocercoids of* Schistocephalus solidus *in sticklebacks collected May to August*

(Figures to nearest whole number)

Month	No. of fish examined	No. of larvae of each size range per fish: >2 cm.* Max.	>2 cm.* Min.	>2 cm.* Mean.	2–1 cm.* Max.	2–1 cm.* Min.	2–1 cm.* Mean	<1 cm.* Max.	<1 cm.* Min.	<1 cm.* Mean	Total no. of larvae of each size: >2 cm.*	2–1 cm.*	<1 cm.*
May	22	6	1	4	8	0	1	2	0	<1	84	29	7
June	11	5	2	4	0	0	0	1	0	<1	41	0	1
August	13	2	0	<1	45	0	19	130	0	39	7	244	434

* Measured in contracted condition.

(*b*) *Morphology*. The anatomy of the larvae has been studied histologically by Smyth (1946). Segmentation becomes evident in larvae of 6–9 mm. and the rudiments of the genitalia appear in about the 8–9 mm. stage (Text-fig. 14). The genital primordia appear as small bands of nuclei in the mid-line. These are best developed in the middle proglottids, becoming less well developed anteriorly and posteriorly. In 17 mm. larvae (Text-fig. 15) these become dumbbell-shaped and by the time the larvae reach about the 32 mm. stage (Text-fig. 16) all the primordia of the genitalia are laid down. In transverse sections, most of the genitalia may be located without difficulty. The main tubular organs, vagina, uterus and vasa differentia, appear as thick-walled tubes (Text-figs. 3, 4) with narrow lumina; the genitalia openings are fully formed. The smaller tubular organs, vasa efferentia, yolk ducts, cannot be located histologically. The shell gland complex is particularly prominent, more so than in the adult



Text-figs. 12–16. Various sized plerocercoids showing development of genitalia and proglottids. Whole-mount preparations.

Text-fig. 17. 65 mm. adult worm. Whole-mount preparation. Note abnormal segmentation in posterior region.

worm, and consists of a compact mass of cells enclosing the future ootype region of the uterus. The ovary (Text-fig. 4) is poorly developed and consists of a loose network of deeply staining cells. The testes and vitelline glands are only recognizable by their positions relative to the other genitalia; they consist of clusters of undifferentiated cells containing darkly staining nuclei and little apparent cytoplasm. This great affinity of nuclei in the larval genitalia for basic stain is one of the characteristic features of the plerocercoid; for the most part, nuclei stain so heavily that it is not possible to make out their detailed structure.

V. DISCUSSION

The structure and life cycle of this cestode presents several unusual features. The most striking, perhaps, is the highly organized condition of the plerocercoid as indicated by the complete external and internal segmentation and the development of the well-formed genital primordia. This condition is unique among cestodes, although it is approached by the plerocercoid of *Ligula intestinalis* which, however, shows only superficial pseudo-segmentation and genital primordia of a lower degree of development. The rapidity with which the plerocercoid of *Schistocephalus* reaches maturation in the bird gut—36 hr. —is likewise unequalled among cestodes; though, again, *Ligula intestinalis* approaches this condition, becoming mature in 63 hr. (Smyth, 1949).

Both these cestodes share a peculiar morphological feature, namely the absence from the scolex of the lateral bothria typical of most of the other members of the family Diphyllobothriidae. This feature suggests that attachment of the adult to the intestinal wall of the definitive host would not be possible, and *ipso facto* imposes the necessity of rapid maturation on the plerocercoid in order to ensure that normal development culminating in oviposition will take place in the short time that the worm is passing down the gut.

It is interesting to note that forms such as *Diphyllobothrium dendriticum* or *D. ditremum* (often found associated with *Schistocephalus* in the stickleback) which have adult lives of up to 105 days (Hickey & Harris, 1947), have well-developed bothria which can act as efficient organs of attachment, even becoming firmly attached to the wall of culture tubes during cultivation *in vitro* (Smyth, 1947). The relationship between the duration of intestinal adult life and the presence or absence of bothria seems clear.

The enormously high parasite/host weight ratio of the plerocercoid of *Schistocephalus* is only surpassed by *Ligula intestinalis* where, according to Plehn (1924), the weight of the plerocercoid may equal the weight of the fish. In this connexion, it is surprising to find that whereas the presence of *Ligula* plerocercoids produce castration in fish, no such effect is produced by the plerocercoids of *Schistocephalus*. Kerr (1948) showed that in roach parasitized by *Ligula* the basophiles in the pituitary were much reduced in size and granulation. Since these cells control the gonad-stimulating hormone, there seems little doubt that the reduction of the gonads in parasitized fish is due to the failure of the pituitary to produce the necessary hormones. In sticklebacks infected with *Schistocephalus*, on the other hand, Kerr found that the effect was much less severe—no difference between the pituitaries of normal and parasitized fish being found. There was evidence of some retardation of spermatogenesis, although large numbers of spermatozoa were found. In the ovary, however, there was a very marked degree of atresia in the largest eggs. Kerr believes that 'this is a case of much more balanced parasitism in which the pituitary of not noticeably altered appearance can carry on the gonads towards the end of their maturation at least'.

The high glycogen content of the plerocercoids presents a further unusual feature. Hopkins (1950) found that the glycogen content was $16{\cdot}2 \pm 0{\cdot}87\%$ (wet weight) which is equivalent to 47–54% dry weight. Such a high degree of carbohydrate food reserves explains how larvae can live in non-nutrient media for long periods under starvation conditions. At room temperatures, plerocercoid larvae may live *in vitro* for periods up to 9 months: at 40° C. in suitably buffered non-nutrient media, the food reserves are sufficient to allow complete maturation to take place (Smyth, 1950).

Schistocephalus appears to occur normally in members of the stickleback family (*Gasterosteus*, *Pyogosteus*), and its occurrence in other hosts is probably accidental. In this respect it differs markedly from *Ligula*, the plerocercoids of which will develop in a very wide range of fish hosts. Cooper (1918) lists seventy species of teleosts from which *Ligula* has been reported.

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EXPLANATION OF PLATE V

Fig. 1. Adult strobila—middle region. Paracarmine.

Fig. 2. Part of transverse section showing muscle arrangements. Bouin; iron haematoxylin.

Fig. 3. 32 mm. plerocercoid—middle region. Paracarmine.

Fig. 4. Partly opened stickleback infected with plerecercoid, which has mostly emerged to the outside; some relaxation has occurred.

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STUDIES IN THE TRANSMISSION OF *PLASMODIUM MALARIAE* BY *ANOPHELES* MOSQUITOES

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(With 1 Figure in the Text)

From a study of the literature, it is clear that less is known about the transmission of *Plasmodium malariae*, either in nature or in the laboratory, than about the other species of human malaria parasites, not excepting *P. ovale*.

Since the introduction of malaria therapy over 30 years ago, malariologists in many institutes in various countries have maintained three, and sometimes four, species of human malaria parasites. With the exception of *P. malariae*, no insurmountable difficulties have arisen in maintaining for very long periods each of these species of parasites by passage through man and mosquito. One example may be quoted. A strain of *P. vivax*, established at Horton in 1925, has been maintained until the present time (1950), and many hundreds of batches, comprising thousands of mosquitoes, have been infected. *P. falciparum* has also been maintained for long periods by a few workers, but, because this species cannot be used with the same degree of safety for therapy purposes, the passage from man to mosquito and mosquito to man has not extended continuously over such long periods as it has with *P. vivax*.

It has been our experience with numerous strains of species of human malaria parasites that our indigenous *Anopheles atroparvus* is an efficient carrier of all the strains of *Plasmodium vivax* which we have used. These include tropical strains from Africa and India and several from Eastern Europe. On the other hand, we have found that while *Anopheles atroparvus* is susceptible to Eastern European strains of *Plasmodium falciparum*, from Italy, Roumania and Sardinia, it is completely refractory to some tropical strains, including strains from East and West Africa and India. However, an Indian strain of *Anopheles stephensi* (type) was found to be highly susceptible to infection with the same West African strain of *Plasmodium falciparum* which failed to infect *atroparvus*. The American *Anopheles quadrimaculatus* was also found to be moderately susceptible to the West African strain. This is remarkable, because both *A. atroparvus* and *A. quadrimaculatus* presumably belong to the *A. maculipennis* complex.

We have also found that *A. atroparvus* is susceptible to *Plasmodium ovale* which, although it has been reported on various occasions from other parts of the world, is, we believe, a parasite of tropical Africa.

Our studies on *P. malariae* at this laboratory began in 1928, when we obtained a strain from Prof. Kirchbaum in Hamburg. During the past 20 years we have worked with nine strains of *P. malariae*. Five were from Africa, one from Holland, one from Roumania, one from South America and one was of unknown origin. It was not until we had been working with *P. vivax* for 5 years that a strain of *P. malariae* was introduced into this laboratory. Unaware of the difficulties of infecting *Anopheles* with *Plasmodium malariae*, we proceeded to feed batches of *atroparvus* on a case in whose blood there were numerous *P. malariae* parasites and what we believed to be gametocytes. We expected that when some of the mosquitoes were dissected several days later, at least a few mosquitoes would show infection. This, however, was not so, and it was only after many batches had been fed on numerous human cases that one or two mosquitoes from a batch of a hundred or more were found to be infected, and then with *scanty oocysts only*.

Table 1 shows the number of attempts made to infect a batch of mosquitoes and the result of the dissections.

Although many mosquitoes from these batches were kept for over a month at 75° F. none showed sporozoites in the salivary glands and none of the patients bitten by them developed infection. This seemed to suggest either that the odd infected mosquitoes had been dissected prematurely or that the oocysts had degenerated before reaching maturity. The number of gametocytes seen in blood films was very few by comparison with *P. vivax* and *P. falciparum*. In many cases no definite male parasites were detected, while in others what we believed to be gametocytes were seen sometimes, though but rarely, in numbers as high as 300 per cu.mm. What were believed to be females were found more frequently than males, but these parasites very closely resemble the mature schizont before division of the nucleus has occurred, and in our experience it is not always possible to distinguish a female gametocyte in a stained film from a fully grown asexual parasite.

With *P. vivax* and *P. falciparum*, host suscepti-

bility is not difficult to determine because human gametocyte carriers, often exceeding 1000 or more gametocytes per cu.mm., are readily available at one time or another. With *P. malariae*, however, typical gametocytes seldom exceed 100 per cu.mm., and it is therefore difficult to state with certainty whether a given species of mosquito is a poor carrier, or whether the gametocytes are immature or are too few to produce infection in a susceptible host.

Green (1929), working in Malaya, carried out a series of experiments with *Anopheles maculatus* as the vector, which he fed on cases of *Plasmodium vivax*, *P. falciparum* and *P. malariae*. He concludes '*Anopheles maculatus* is susceptible to infection with *Plasmodium falciparum* and *P. vivax* and is capable of transmitting both sub-tertian and benign tertian malaria'. With *P. malariae*, however, although some mosquitoes became infected with oocysts none of these developed to sporoblast formation, and no sporozoites were found in the salivary glands. Three hundred gametocytes per cu.mm. were the highest number found, the sexes being about equal in number It is of interest that in one series he succeeded in producing infection (oocyst stage only) in 33% of mosquitoes with an average of 1·5 oocysts per gut when there were only 168 gametocytes per cu.mm., yet he had negative results in another series when the gametocytes were 300 per cu.mm. This variability may have been due to immaturity of the gametocytes in the negative series, but his figures suggest that about 200 gametocytes per cu.mm. represent the border-line of infectivity with this species of malaria parasite and the species of mosquito tested (*Anopheles maculatus*).

Green's results are closely similar to our earlier findings between the years 1928 and 1945 which we have already quoted. His work was carried out using patients infected in nature, and therefore the patient on whom the mosquitoes were fed was infected by sporozoites through the agency of a mosquito. All our patients were infected by direct blood inoculation, usually intramuscularly but sometimes intravenously. Many workers claim that as the result of repeated passages of the parasite of *Plasmodium vivax* by direct blood inoculation, gametocytes diminish in numbers with each successive passage of the parasite and eventually disappear altogether. Whether or not this also occurs with *P. malariae* we are unable to say, but it is the case that the few mosquitoes we succeeded in infecting during the period 1928–45 occurred when the strain was only a few passages removed from the originally infected patient.

Compared with the many successful transmissions from man to mosquito and mosquito to man with *P. vivax* and *P. falciparum*, there have been very few successful transmissions with *P. malariae*. At least nine species of Anopheles have, however, been experimentally infected in various parts of the world, and the infection transmitted to man. Other workers have infected Anopheles as far as the stage of oocyst formation, and some even up to the point of finding sporozoites in the salivary glands, but have not transmitted the infection to man which is, we venture to suggest, essential before a species of mosquito can be labelled a carrier (Table 2).

Table 1. Anopheles maculipennis *var.* atroparvus

(Out of nineteen batches of mosquitoes fed on various patients between 1928 and 1945, six batches became infected.)

No. of batch	No. of mosquitoes dissected	No. of mosquitoes infected	No. of oocysts per stomach	Percentage positive
1	12	2	1, 2	17
2	107	2	1, 2	2
3	52	4	2, 2, 1, 1	8
4	11	1	5	9
5	57	1	5	2
6	20	2	1, 1	10

In 1947 we started a colony of *Anopheles stephensi* (type) kindly sent to us by Lt.-Col. Jaswant Singh, Director, Malaria Institute of India. This afforded us an opportunity to experiment with this species of Anopheles and *Plasmodium malariae*. The strain of parasite used in our experiments was obtained from a seaman who contracted his disease in West Africa and was kindly sent to us by Dr D. R. Seaton of the Liverpool School of Tropical Medicine. Table 3 shows the number of attempts made to infect a batch of mosquitoes, the results of dissections and the number of patients infected.

Our first success in infecting a patient was with batch no. 4 (Table 3). After the mosquitoes had been incubated at a temperature of 78° F. for 25 days they were fed on a patient daily for 3 days, the same insects being used each time. Forty-nine bites were recorded and following the last feeding, the glands of all the surviving mosquitoes were dissected, but no sporozoites were seen. The number of sporozoites injected into the patient could not have been very numerous; even so, fever and parasitaemia developed, the incubation period being 46 days (parasites and fever). This is also the case with batch no. 6. Only

Table 2. *Records of experimental infection of various species of* Anopheles *with* Plasmodium malariae

Author	Year of publication	Species of mosquito	Percentage of mosquitoes infected	Incubation period in mosquito (in days)	No. of males and females per cu.mm.	No. of patients infected	Incubation period in man (in days) (F = fever, P = parasites)	Country
Averbouch	1930	*A. sacharovi*	—	—	—	1	32	Russia
de Buck	1935	*A. atroparvus*	27	15	12	4	24–28	Holland
Mackerras & Ercole	1948	*A. punctulatus*	—	28	—	1	24	Australia
Marotta & Sandicchi	1939	*A. maculipennis* (*messeae*)	—	24	—	2	23–29	Italy
Mer	1933	*A. elutus* (*sacharovi*)	39	30	100 males and females	1	31	Palestine
Mer	1933	*A. elutus* (*sacharovi*)	15	27	56 males and females	1	26	Palestine
Mer	1933	*A. elutus* (*sacharovi*)	22	28	120 males and females	1	28	Palestine
Young & Burgess	1947	*A. freeborni*	—	32	67 males and females	1	59	U.S.A.
Hylkema	1920	*A. ludlowi*	20	11–13	—	1	12	Dutch East Indies
Siddons	1944	*A. culicifacies*	34	14 at 80° F. 22 at 70° F.	300–600	1	36	India
Boyd & Stratman Thomas	1933	*A. quadrimaculatus*	33	30	—	3	27, 42, 32	U.S.A.
Boyd & Stratman Thomas	1936	*A. quadrimaculatus*	25, 22	34	1000 males and females	2	37, 28	U.S.A.
Shute & Maryon	—	*A. stephensi* (type)	11	19 (approx.)	—	1	F. 46, P. 46	England
Shute & Maryon	—	*A. stephensi* (type)	4	—	—	1	F. 38, P. 35	England
Shute & Maryon	—	*A. stephensi* (type)	63	18	—	3	(1) F. 32, P. 30 (2) F. 31, P. 31 (3) F. 21, P. 28	England
Shute & Maryon	—	*A. stephensi* (type)	2·5	21 (approx.)	—	1	F. 25 P. 16	England

one out of twenty-three mosquitoes dissected was positive and then with only a single oocyst. On the first day when sporozoites might have been expected to have invaded the glands there were about twenty mosquitoes alive. These were fed daily on a patient over a period of 12 days, the patient receiving 138 bites. Fever and parasitaemia developed, parasites were found on the 35th day and fever occurred on the 38th day. In both these cases it is assumed that infection occurred on the 1st day of biting, although this is not certain.

Batch no. 8 represents our most successful efforts. The patient on whom the mosquitoes fed was infected by direct blood inoculation twice removed from a mosquito-infected case. The mosquitoes were fed on zoites appeared in the glands there was thirty mosquitoes left alive. Three patients were bitten on one or two occasions only, and all three developed fever and parasitaemia, with incubation periods of 28, 30 and 31 days respectively. Blood films (thick and thin), were examined daily for gametocytes from one of these three patients. On the 50th day of parasitaemia scanty female gametocytes, but no males, were found. A batch of thirty-five *Anopheles stephensi* were fed on this day and eight (23 %) were positive, for oocysts only. All the mosquitoes were dissected on the 4th and 5th day after feeding. A single oocyst was found in each of five mosquitoes and in each of three mosquitoes two oocysts.

On the 55th day of parasitaemia and daily until

Table 3. Anopheles stephensi (*type*)

No. of batch	No. of mosquitoes dissected	No. of mosquitoes infected	No. of oocysts per stomach	No. of mosquitoes with sporozoites in glands	Percentage positive	Remarks
1	46	2	3, 2	Nil	4	—
2	23	1	1	Nil	4	—
3	17	1	1	Nil	6	—
4	21	3	2, 2, 2	Nil	14	This batch infected one patient
5	66	1	1	Nil	1·5	—
6	23	1	1	Nil	4	This batch infected one patient
7	34	2	1, 1	Nil	6	—
8	48	24	1, 2, 3, 3, 3, 4, 5, 5, 6, 8, 9	13	56	This batch infected three patients
9	61	1	2	Nil	1·5	—
10	31	9	1, 1, 1, 1, 1, 2, 2, 2, 2	Nil	29	—
11	60	3	1, 1, 1	Nil	5	—
12	340	4	1, 1, 1	1	2·5	This batch infected one patient; from a mosquito-infected case (from a patient in batch 8)

(Out of sixty-eight batches of mosquitoes fed on various patients between 1948 and 1950, twelve batches became infected.)

the patient on the 14th day after the first observance of fever and parasites. No typical male gametocytes were found in thin blood films, but it was believed that one or two males were seen in thick films. Characteristic female gametocytes were found in thin films, and the batch of mosquitoes was fed on speculation and not because the blood picture indicated that mosquitoes should become infected. The mosquitoes were kept at a temperature of 75° F. (24° C.). We were surprised to find such a high percentage of mosquitoes infected and also that one stomach contained nine oocysts, the largest number seen in any of our dissections. We examined thin blood films of the patient, taken on the day the mosquitoes were fed, but failed to find a parasite which conformed to the text-book picture of a typical male gametocyte. On the day after sporo- the 59th day, a total of 340 *A. stephensi* were fed. With the exception of 1 day, the 56th when, a female was found, no recognized gametocytes were seen on any day in either thick or thin films. Of the 340 mosquitoes fed, 158 were dissected. Four (2·5 %) were positive, a single oocyst was found in each of three mosquitoes and one showed sporozoites in the glands; this mosquito bit and infected a patient.

It is not possible to record the extrinsic incubation period for this batch of mosquitoes, because they were fed over a period of several days, and also because we do not know how long the sporozoites had been in the salivary glands, but we believe it was about 21 days. As the patient was bitten on only one occasion by the mosquito with sporozoites in the glands, we do know the intrinsic incubation period was 16 days. On this day very scanty parasites were

found in thick films. The patient had no fever until the 9th day of parasitaemia, thus giving a fever incubation period of 25 days.

This equals Boyd's and Stratman Thomas's record of transmitting quartan malaria through two consecutive human-anopheline passages, with laboratory-bred *Anopheles* mosquitoes and induced human carriers.

It is interesting to record that 5 days after batch 8 was fed, a further batch of several hundred mosquitoes was allowed to feed on the patient. We believed that an opportunity had arisen to produce a large infected batch. Not a single infected mosquito was however found, although 100 were dissected between the 4th and 13th day after feeding and were kept under the same conditions as batch 8. This suggests, either that the gametocytes of *Plasmodium malariae* are transitory in the blood, or that they quickly lose their viability; it seems to us that the former is the more probable. Gametocytes were not found in the blood film of the patient on the day this negative batch was fed, but neither were they on the day when batch 8 became infected. Had our transmission experiments been confined to feeding mosquitoes on patients in whose blood typical male gametocytes were found, we would have expected that some, but not all, of our batches would have been infected. On the other hand, batches became infected by feeding on patients where no gametocytes were found. This is quite beyond our understanding.

Other workers have stressed the difficulty encountered in defining clearly the gametocytes of *P. malariae*. Boyd & Stratman Thomas (1933), discussing the morphology of *P. malariae* gametocytes, state: 'None of the illustrations of this cell given in the standard works of reference at our disposal accurately represent it. Yet the characteristics observed are strikingly similar to those exhibited by the male gametocyte of other species.' We are in agreement with this statement. Despite the success achieved with *P. malariae* our successful transmissions are not the result of feeding mosquitoes on patients whose blood contains a minimum number of male and female gametocytes in a given number to a cu.mm., as is our practice in the case of infections with *P. vivax* and *P. falciparum*. For example, we find that with *P. vivax*, six mature male and six female gametocytes per cu.mm. represents the border-line of infectivity with *Anopheles maculipennis* var. *atroparvus*. With *Plasmodium falciparum*, on the other hand, the border-line of infectivity is in the region of 12 per cu.mm. as noted by Darling in 1910. We have not been able to correlate the number of *P. malariae* gametocytes with the border-line of infectivity. On some occasions we have found male gametocytes far in excess of 12 per cu.mm. and at least an equal number of female gametocytes, yet large batches of mosquitoes fed on the carrier have been negative. On the other hand, we have succeeded in infecting batches when we have failed to find a single typical male gametocyte after a prolonged search in thin films. To us the so-called mystery of Quartan transmission is not that a batch of mosquitoes is negative after feeding on a case in whose blood characteristic male and female gametocytes were present, but that batches of mosquitoes are often found to be infected when no characteristic male gametocytes have been found even to a thousand or more leucocytes. If, therefore, the male gametocyte of *P. malariae* is, as the text-books show, a small edition of the male gametocyte of *P. vivax*, then our results suggest that even if there are not typical forms which cannot be recognized as gametocytes, then less than 6 males per cu.mm. are able to produce infection in some species of Anopheles.

As stated by Brumpt (1949): 'We know that the infection of mosquitoes is favoured not so much by the absolute number of gametocytes in the circulating blood as by certain uncertain "qualities" of these elements. Very beautiful infections of anophelines are obtained at times from patients with few gametocytes; whereas numerous failures have occurred when other subjects were used who seemed better because they were rich in gametocytes. No writer has yet been able to explain these facts.'

James (1910) described the morphology of *P. malariae* gametocytes as follows:

'In their youngest stages the gametes of both sexes appear small, circular parasites with a relatively large amount of chromatin. These young gametes as they grow resemble very closely the schizonts and two forms can be distinguished. One form is more heavily pigmented, with a smaller amount of chromatin, than a schizont of the same size, while the other form stains somewhat lighter and has more chromatin. When the gametes fill the entire erythrocyte they are easily identified, if it be remembered that a full grown parasite in which the chromatin is not dividing, is a gamete. This statement is not to be taken as a definition of a gamete, but only as a method of identification.

'In the adult macrogamete the cytoplasm is a deep blue and contains numerous darker blue granules. The chromatin is granular also, compact, is collected into one or two masses, and never presents the divided appearance seen in the schizont. The pigment is in very heavy masses distributed irregularly throughout the cytoplasm. No trace of the red blood cell is visible. The microgamete is somewhat smaller than the macrogamete, and is a rare form. Like the macrogamete the adult microgamete requires 6 days to attain its full growth. At maturity it is about the size of a red blood cell and is smaller than the female form. At this time it has a very large amount of chromatin, loosely arranged, so

that the threads can be plainly seen. The cytoplasm is stained very lightly and diffusely, sometimes so faintly that it has a hyaline appearance. The pigment, while abundant, is neither so plentiful nor so coarse as in the macrogamete. The microgamete, though rare, when once seen is easily recognized. Attempts to infect mosquitoes, so that further changes in the gametes might be followed, were unsuccessful. Nor was I able to stain any flagellate forms.'

Although many workers in various parts of the world have infected *Anopheles* mosquitoes with *P. malariae* and transmitted the disease to man, no one has, as far as we know, described the day-to-day pattern and character of the pigment in *P. malariae* oocysts from the youngest forms to sporoblast formation. The character and pattern of the pigment in *P. malariae* is so distinctive from *P. vivax*, *P. falciparum* and also *P. ovale*, as to enable species diagnosis in the field to be arrived at with some degree of certainty.

Batch no. 8 of our series afforded us the opportunity of studying the oocysts day by day throughout the extrinsic incubation period up to the invasion of the salivary glands by the sporozoites. These are illustrated in Fig. 1. (After dissection the infected mid-guts were flattened with a cover-slip and fixed in Bles's fluid. Measurements were made after fixation.)

Three-day oocysts. These measure 5 μ. The pigment is nigger brown to black, variable in size and distributed throughout the cyst. There are approximately thirty grains of pigment.

Four-day oocysts. These measure 6–8 μ. The pigment is the same as for 3-day oocysts and is distributed throughout the cyst.

Six-day oocysts. These measure 9 μ. In some the pigment shows a tendency to become clumped but in others it is but little changed from younger oocysts.

Eight- and nine-day oocysts. These measure from 10 to 12 μ. The pigment shows a tendency to move towards the contour of the cyst with considerable clumping. The number of grains of pigment seen are usually less than in younger oocysts.

Eleven-day oocysts. These measure from 16 to 20 μ. The pigment has moved to the contour of the cyst and is markedly clumped, but some have a few grains away from the main mass of clumped pigment.

Twelve-day oocysts. These measure from 24 to 26 μ. The pigment is concentrated at the contour of the cyst and is markedly clumped.

Thirteen-day oocysts. These measure between 27 and 34 μ. Because of the multiple division of the nuclei, pigment cannot always be seen, but when visible it is nearly always situated at the periphery of the cyst and is clumped.

Seventeen-day oocysts. These measure from 27 to 39 μ. In many oocysts no pigment can be seen, but where it is visible it is situated at the periphery of the cyst.

Twenty-one-day oocysts. These measure from 37 to 44 μ. Sporozoites fill the cysts, pigment can rarely be seen, but when ripe cysts rupture on pressure from a cover-slip, large black grains of pigment can be seen.

Although female gametocytes measure 7–8 μ, the youngest oocysts measure only 5 μ. The largest oocysts we have seen measure 44 μ, but some may rupture and release sporozoites when they are only 37 μ. From Fig. 1 it will be seen that from the 7th day onwards some oocysts grow far more rapidly than do others. This, of course, also applies to *P. vivax*, *P. falciparum* and *P. ovale*.

Some workers have described the colour of the pigment of *P. malariae* oocysts as dark brown, black and even light coloured. We would describe the colour as nigger brown to black but on occasion in large oocysts, where clumping of the pigment has occurred, one sees a golden brown tinge which appears as a diffused smudge as though some grains of pigment have disintegrated.

The largest number of grains of pigment seen in an individual oocyst was 36. This was in 5-day oocysts. When the cyst has reached the 6th day of growth some clumping of the pigment begins, and consequently the number of individual grains seen are fewer, and, as will be seen in Fig. 1, the pigment varies considerably in size; Hylkema (1920) states that the pigment fades and diminishes when the cysts grow larger, and then it changes from rough to fine. We believe that there is no diminishing in size of the individual grains of pigment, but that the reason why less pigment is seen as the oocysts grow larger is that the multiple division of the nuclei partially or completely obscures the pigment. Brumpt (1949) states that the number of grains of pigment in young *P. malariae* oocysts varies between 6 and 12 grains. The number may vary with the strain of parasite and even with the age of the oocyst, but in our experience there are about 30–50 grains of pigment in a female *P. malariae* gametocyte and most, if not all, are carried over to the oocyst stage and can be seen up to about the 6th day of growth, before clumping and multiple division of the nuclei occurs.

The membrane of the cyst is thick and easily seen in unstained and stained specimens, but is not conspicuously thicker than in *P. vivax* or *P. falciparum* oocysts. Many workers have pointed out that in *P. malariae* oocysts the pigment is invariably concentrated in a mass at the periphery. This is so with oocysts at and beyond mid-way through the developmental cycle, from the 11th day onwards. Between the 3rd and 10th day the pigment is

scattered all over the cyst, and is very irregular in both size and shape.

The sporozoites

Our findings agree with those of other workers in that the sporozoites of *P. malariae* are longer and broader than in *P. vivax* and *P. falciparum*. In *P. malariae* they measure up to 14 μ as against 11 and 12 μ in *P. vivax* and *P. falciparum*. We believe, too, that there is more chromatin in *P. malariae* compared with the other species, and often it consists of a thick deeply stained bar occupying as much as one-third the length of the sporozoite.

DISCUSSION

Although we have so far failed to transmit *P. malariae* to man by the bites of *Anopheles maculipennis* var. *atroparvus*, we have succeeded in doing so with *A. stephensi*. de Buck (1935) and others have infected *atroparvus* and transmitted the disease to man.

Good gametocyte carriers are rare, and therefore it is not easy to carry out comparative feeding experiments with several species of *Angpheles*. In our experience, however, *A. stephensi* is a much more efficient carrier of *Plasmodium falciparum* than is

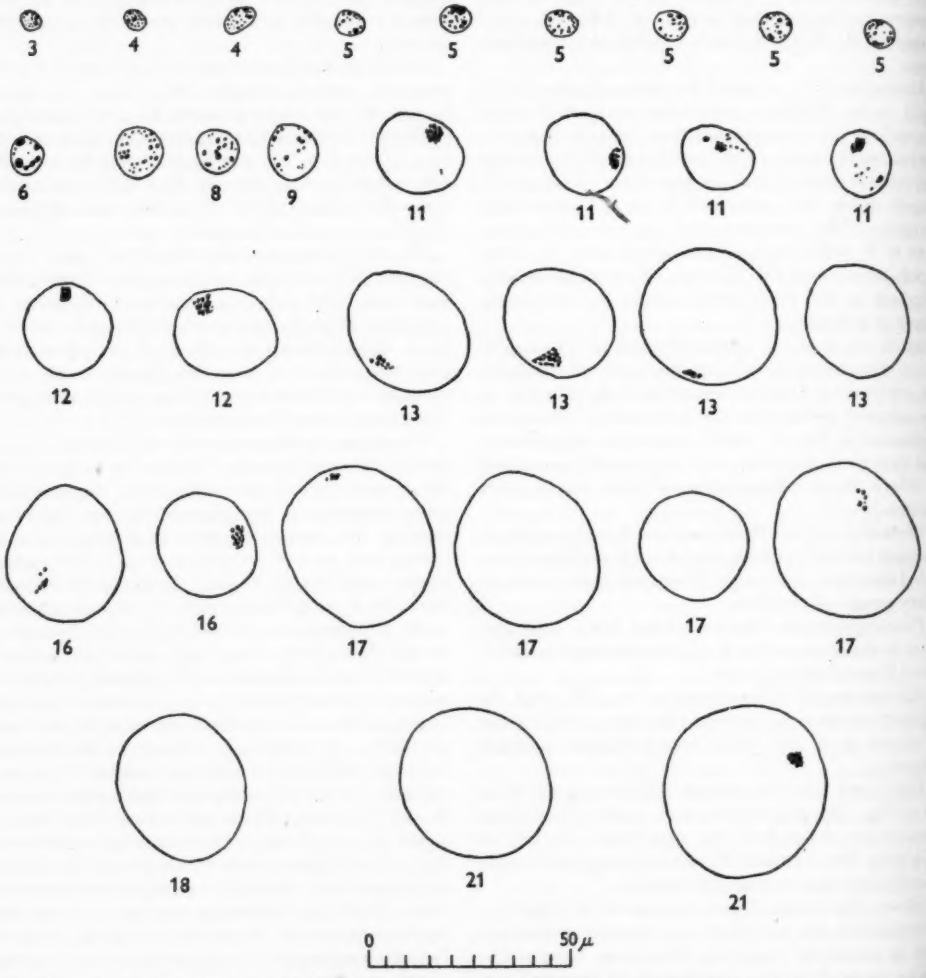

Fig. 1. Character and arrangement of the pigment in oocysts of *P. malariae* as seen in *Anopheles stephensi* (type). The numbers denote age of the oocysts in days.

atroparvus and this probably also applies to *P. malariae*.

Our findings agree with those of some other workers in that mosquitoes fed on one day may become infected, but another batch of the same species of mosquito fed on the same patient a day or two later may be negative, even though no anti-malarial drugs have been given. To some extent this applies to *P. vivax* but not, in our experience, to *P. falciparum*.

According to Ross (1910) there are about 1000 sporozoites in an oocyst. Not all the sporozoites from rupturing oocysts enter the salivary glands; for example, large numbers can be seen in sections of the thoracic muscles. It is, therefore, probable that at least some of our patients received less than 1000 sporozoites.

Parasites may be found in thick blood films in very small numbers for as long as 10 days before fever begins. It seems to us likely that if patients were infected by very large numbers of sporozoites, as is possible in *P. vivax* and *P. falciparum*, the intrinsic incubation period may be shortened considerably, because the number of merozoites released from the liver stage may be in sufficient numbers to produce fever after only one or two cycles in the peripheral blood. When, however, infection is produced by only a few sporozoites, several generations of erythrocytic cycles may be required before parasites are sufficiently numerous to produce clinical symptoms or to be demonstrable in thick or thin films. Unfortunately, we have not been able to infect a patient with very large numbers of sporozoites, and until this has been done it will not be possible to determine the minimum incubation period.

P. malariae oocysts show pigment which differs greatly from those of other human Plasmodia. Up to about the 6th day the pigment is extremely coarse and resembles *P. falciparum*. Unlike the latter, however, the pigment is scattered all over the cyst and the individual grains vary in size. After the 6th day there is a marked clumping of the pigment which is concentrated at the periphery of the oocyst. It is rare to see a large oocyst which fails to conform to this description, and it is considered that field workers should experience very little difficulty in recognizing *P. malariae* oocysts in dissections of mosquitoes caught in nature. When the oocysts are mature (sporoblast formation) pigment cannot always be seen, but this also applies to the other species of human malaria.

The findings of field workers are in harmony with the results obtained by workers in the laboratory who have studied the gametocytes and their output as seen in patients undergoing malaria-therapy. A study of the literature shows that at the present time very little is known about *P. malariae* gametocytes, their morphology, time of appearance in the peripheral blood in relation to the onset of the disease and their virile longevity after appearing in the blood.

Among twenty-four cases in Table 2 which were infected experimentally by mosquito bites, in two cases the intrinsic incubation period was less than 20 days. In all the others the incubation period varied between 21 and 59 days. That this represents the pre-patent period (first invasion of erythrocytes as distinct from clinical symptoms), is doubtful. Now that the fate of the sporozoite in *P. vivax* and *P. falciparum* has been solved by Shortt and his colleagues there can be but little doubt that the same pre-erythrocyte cycle in the liver of man occurs also in *P. malariae*. If, then, the incubation period in *P. malariae* averages about 30 days, it may be thought that the liver phase in this species is longer than in *P. vivax* and *P. falciparum*. It may be a few days longer, but it is probably not so long as the figures would indicate because it is likely that when the infection by sporozoites is a light one, several generations of the erythrocytic cycle may be necessary before parasites are sufficiently numerous to produce clinical symptoms.

SUMMARY

P. malariae was first used for therapy purposes at Horton in 1928.

Between 1928 and 1945, nine strains have been used. Most of these were from Africa, but some were from Holland, Roumania and South America. The strain from Holland was probably not indigenous to that country.

All transmission experiments carried out between 1928 and 1945 were with *Anopheles maculipennis* var. *atroparvus*. Nineteen separate batches of mosquitoes were fed on patients in whose blood parasites believed to be gametocytes were seen. In only six of these batches were any of the mosquitoes found to be infected and then with only scanty oocysts; sporozoites were never found. No patients were infected by the bites of any of these mosquitoes.

In 1947 a colony of *A. stephensi* (type) was established.

From 1948 to 1950, sixty-eight batches of *A. stephensi* were fed on patients in whose blood *Plasmodium malariae* parasites were present. Scanty gametocytes were found in some of these patients, and on occasion mosquitoes which fed on them became infected, but in others no infection occurred.

Other batches of mosquitoes did become infected after feeding on patients in whose blood no typical gametocytes were found even after a prolonged search. Out of sixty-eight batches of mosquitoes fed, infection was seen in twelve batches, in varying degree, but never with more than nine oocysts in an

individual mosquito. Two consecutive human anopheline passages with laboratory-bred *Anopheles* mosquitoes (*A. stephensi* type) and induced human carriers are reported.

Despite our success in infecting and transmitting *Plasmodium malariae* parasites to man by the agency of mosquitoes, we are unable to give any concise figures of the number of gametocytes per cu.mm. which must be present to produce infection in the mosquito.

The extrinsic and intrinsic incubation periods observed by us compare favourably with the findings of Boyd & Stratman Thomas (1936). The intrinsic incubation period varies greatly between individual patients.

The time required for the complete development of the pre-erythrocytic cycle is discussed. It is pointed out that this is probably not so long as the intrinsic incubation period might suggest, because it seems probable that several generations of the erythrocytic cycle are necessary before the number of parasites is sufficiently high to produce clinical symptoms.

The character and arrangement of the pigment in developing oocysts are diagnostically different from those observed in all other known species of human Plasmodia. Workers in the field who become thoroughly acquainted with the character and arrangement of the pigment in developing *P. malariae* oocysts should have no difficulty in determining this species of parasite in mosquitoes caught in nature.

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THE QUANTITY AND DISTRIBUTION OF THE CILIATE PROTOZOA IN THE LARGE INTESTINE OF THE HORSE

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(With 4 Figures in the Text)

In 1930, Hsiung described thirty-seven species of Ciliata and three species of Suctoria which occurred in material from the large intestine of forty-six horses, to which he added eleven species of Ciliata described by earlier investigators. The object of the present work was to determine the number and distribution of the Ciliata in the large intestine of the horse.

METHODS

(1) *Collection of material*

Material was obtained from horses killed for human consumption. The horses had access to water only for at least 24 hr. before killing, but their earlier history was unknown. Although this source of material was not very satisfactory, it served to develop a method of separating and enumerating the Protozoa. Later, material was obtained from two horses known to have been feeding up to the time of slaughter. The large intestine was divided into parts named as follows: caecum; colon I, corresponding to the right ventral colon (Sisson, 1938); colon II or the left ventral colon; colon III or the left dorsal colon; colon IV or the right dorsal colon. The small colon was divided into anterior and posterior parts. The anterior part was 1–2 ft. long and its contents were relatively fluid; the remaining posterior part contained formed faeces. Faecal samples were taken from the rectum. The guts of the horses were available about 20 min. after killing. The large intestine was tied off into the regions already described. The contents of each part were emptied into buckets for weighing, transferred to a metal bath and mixed by hand. Glass jars of 300 ml. capacity were filled with the digesta and taken back to the laboratory.

Samples from each part of the gut were treated as follows:

(*a*) Two lots of 10 g. each were weighed and washed into specimen tubes with about 40 ml. of 10% formalin; the tubes were corked and kept for examination.

(*b*) 10 g. was weighed in a porcelain basin and dried in an oven at 60° C. to constant weight.

(*c*) The pH of each sample was estimated with B.D.H. Universal Indicator.

(2) *Separation of the Protozoa*

A diagram of the separation procedure is shown in Fig. 1. A 10 g. sample was emptied into a sieve, the tube rinsed, and the material sieved by agitation for 5 min. in about 200 ml. of water. The sieve was then allowed to drain and the wash water made up to 300 ml. The sieve consisted of a piece of bolting silk stretched tightly across the end of a metal tube 10·5 cm. in diameter and 20 cm. long. The mesh size of the silk was 44 to the inch, which corresponded to an aperture of about 0·5 mm. square. This size of mesh was large enough to allow most of the ciliates and other particles of similar size to pass through, thus separating them from the bulk of the debris. In several experiments the residue on the sieve was washed a second and a third time and then examined microscopically for the presence of ciliates.

(3) *Preparation of suspensions for counting*

The 300 ml. suspension was emptied into a beaker. After thorough stirring, 5 or 10 ml. lots, depending on the density of the suspension, were immediately run into centrifuge tubes. Pipettes with an internal diameter at the tip of not less than 2 mm. were used. In this way there was no impaction of particles at the tip of the pipette and the suspension flowed at an even rate.

The material was centrifuged for 2 min. in a hand centrifuge. Frequent examination of the supernatant showed that this procedure was sufficient to throw down all the ciliates. The bulk of the supernatant was withdrawn leaving the deposit in about 1 ml. To this was added glycerine and a few drops of acid methyl green, and the volume made up to 2 or 5 ml. depending on the counting procedure that was used subsequently. Staining made it easier to distinguish the Protozoa. The addition of glycerine was found to be essential; it increased the viscosity of the fluid and so prevented the rapid sedimentation of the larger ciliates. A suitable viscosity was obtained when the suspension contained about 50% glycerine.

(4) *Counting procedures*

(*a*) *Ruled slide*

This was an ordinary 3 by 1 in. microscope slide with its central area ruled into 0·5 mm. squares.

A 0·2 ml. graduated pipette was used to withdraw fluid from the 2 ml. of concentrated suspension. A drop of 0·1 ml. was placed in the centre of the counting slide and covered with a $\frac{7}{8}$ in. square cover-glass. The material was examined under a $\frac{2}{3}$ in. objective, and all the ciliates were counted.

(*b*) *Sedgwick-Rafter cell*

A counting cell of 1 ml. capacity was made with measurements 50 × 20 × 1 mm. The method of filling the cell and applying the cover-glass was important. Gilbert (1942) concluded that a volumetric error was likely to appear if the cell was not filled with a volume measured from a pipette. Following this procedure, a 1 ml. pipette was used to deliver a constant volume into the cell. Further, in placing the cover-glass, it was found necessary to rest one edge on the cell and gently lower the other with the point of a needle. If the cover-glass was simply made to glide into position the ciliates were no longer distributed at random.

A glass disk, masked with black paper so as to present a central open square, was inserted into the eyepiece of the microscope. Since the size of the square was exactly 1 mm. when projected on to the stage of the microscope, one field of vision covered 1 cu.mm. of fluid in the cell. The ciliates in 400 fields were counted and in this way a total count represents the number of ciliates in 400 cu.mm. of the 5 ml. of concentrated suspension in the centrifuge tube.

DETAILED ANALYSIS OF PROCEDURE

(1) *Accuracy of the counting technique*

(*a*) The ruled slide was used in the early part of this work, but the occurrence of unexpectedly large differences between counts from the same suspension showed that this method was probably unreliable. The distribution of the ciliates on the slide was estimated by counting the numbers in 484 squares and by applying a Poisson calculation to the result (Snedecor, 1946). This was done in four consecutive counts of not less than 1000 ciliates, and the values for P were found to be greater than 0·05, showing that the ciliates on the slide did, in fact, follow a random distribution. Yet in eighty-three paired comparisons from different samples statistically significant differences occurred on thirty-one occasions. The cause of these discrepancies was not clearly established: it may have been connected with the small volume (0·1 ml.) sampled for counting, but was not a counting error. The slide was eventually abandoned in favour of the Sedgwick-Rafter cell.

It should be mentioned that, since the ciliates followed a Poisson distribution on the slide, the square root of the total count was taken as an estimate of the standard error of random sampling. Twice this value provided an estimate of the limits of error at the P 0·05 level of significance. The standard error of the difference between two counts was calculated as the square root of the sum of the counts. The observed difference between two counts was considered to be statistically significant if it exceeded twice the standard error of the difference.

(*b*) The accuracy of counting ciliates with the Sedgwick-Rafter cell was tested in a similar manner. Tests for agreement between observed and expected frequencies were applied to five counts that ranged from 761 to 1664 and the values for P were all greater than 0·05.

Further tests were carried out with samples from different parts of the gut. It was possible that differences in the composition of the samples might influence the distribution of ciliates in the cell; particularly was this suspected in samples from the small colon and rectum, where the material was more concentrated and in which the ciliates were fewer. The results, which are given in Table 1, justified the conclusion that the counting technique was likely to be reliable over the whole range of samples.

Table 1. *Results of tests for random distribution on samples from each part of the gut of horse no.7 (Sedgwick-Rafter cell)*

Sample	Total count	Test of agreement between observed and expected frequencies χ^2	n	P
Caecum	919	1·1446	7	<0·99
Colon I	1262	4·7760	9	<0·8
Colon II	930	9·6237	7	<0·2
Colon III	1378	9·0597	9	<0·3
Colon IV	500	5·0011	6	<0·5
Small colon anterior	449	2·5441	6	<0·8
Small colon posterior	478	1·2516	6	<0·95
Faeces	470	5·5373	6	<0·3

(2) *Errors of sampling at different stages in the separation of the Protozoa*

The various stages in the procedure are shown in Fig. 1. It will be seen that variations in the subsampling may occur between jars (A, B, C and D); between 10 g. lots (A_1 and A_2); between tubes (T_1–T_4) and finally between duplicate counts from the same tube sample.

The values for duplicate counts on samples from different tubes are set out in Table 2. Test samples I and II were more concentrated than the others, and in eight comparison counts there were three significant differences. It was suspected that a counting error was partly responsible for the differences since the ciliates were difficult to distinguish when present in such high concentration.

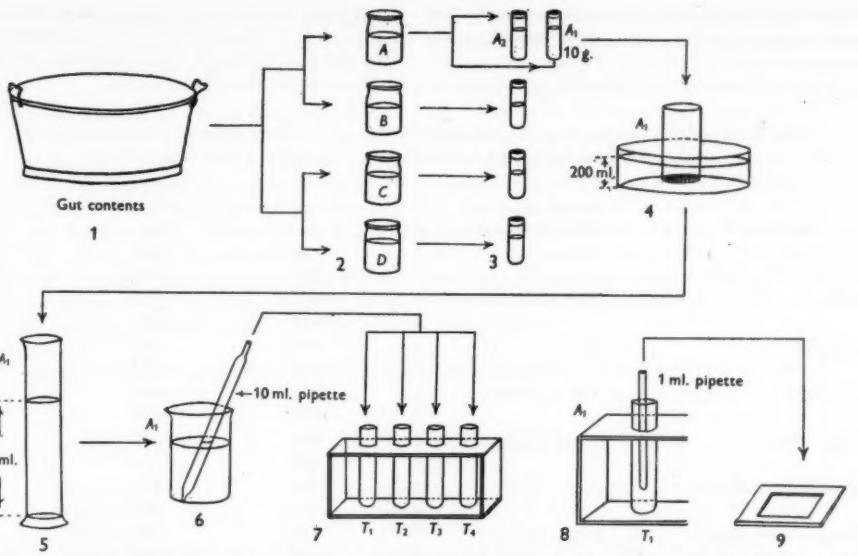

Fig. 1. Diagram of method and apparatus used to separate and enumerate the ciliates in material from the large intestine of the horse: (1) container for mixing digesta; (2) jar samples (300 ml.); (3) formalized samples (10 g.); (4) sieve for separating ciliates; (5) ciliate suspension in measuring cylinder (300 ml.); (6) suspension transferred to beaker (300 ml.); (7) 10 ml. samples in centrifuge tubes; (8) suspension prepared for counting by addition of glycerine and acid methyl green; (9) Sedgwick-Rafter cell of 1 ml. capacity.

Table 2. *Results of duplicate counts on a series of tubes from test samples I–VII*

Test sample	Tube	Count 1	Count 2
I	1	1624	1639
	2	1741	1664
	3	1762	1624
	4	1673	1603
	5	1766	1645
II	1	1402	1500
	2	1373	1525
	3	1355	1415
III	1	723	761
	2	776	767
	3	731	748
IV	1	229	256
	2	246	238
	3	214	235
V	1	761	763
	2	730	732
VI	1	721	735
	2	732	757
	3	775	752
	4	841	767
VII	1	789	774

In test samples III–VII, the suspensions were made less concentrated, and in thirteen duplicate counts none was significantly different. In all subsequent counts an attempt was made to adjust the concentration of the suspension in the tube to give a count of about 1000. This could not always be achieved when, for example in the faeces, the proportion of food residue was high relative to the numbers of ciliates in the sample.

Tests for significant differences between tube samples in each series corresponding to test samples I–VI were carried out by comparing each count in the series with the count for tube 1 of the same series. Using the values for the first count, it was found that in fourteen comparisons there were four significant differences, three of which occurred in the highly concentrated sample I. When the values for the second count were used there were no significant differences.

Further comparisons between tubes are shown in Tables 3 and 4, which contain the results of counts from two well-fed horses (nos. 7 and 8). Altogether there are forty-six independent comparisons between tube samples, three of which are significantly different (Table 3, colon IV A_2; Table 4, colon I C and small colon anterior).

Comparisons between two 10 g. lots, A_1 and A_2, taken from the same jar sample A, are shown in Table 3. When the counts for tube 1 of each series are compared, no significant difference is shown except for the faeces. A difference in the faecal sample might be expected since, owing to its dry, compressed nature, no mixing was possible when

samples were being weighed. Hence it was concluded that subsamples A_1 and A_2 were taken from the same population.

Comparisons between jar samples are shown in Tables 3 and 4. In Table 3 if the counts for tubes 1 of A_1 and B are compared, four out of the six comparisons are significantly different. In Table 4 the results of counts for four jar samples are shown. If the counts for tube 1 of each sample in each series are compared, the differences are often larger than would be expected by chance. These results indicate that the error of sampling is likely to be influenced by factors in the technique at the first stage, when the jars are filled from the bath. The digesta were always mixed by hand in the bath before the jars were filled, but this did not eliminate the occurrence of significant differences between jar samples.

Table 3. *Results of counts from all samples of horse no. 7. Comparisons between subsamples A_1 and A_2 from jar sample A and between jar samples A and B are shown*

Horse no. 7	Concentration of counting suspension	Tube	Sample A_1	Sample A_2	Sample B
Caecum	Nil	1	848	843	985
		2	919	854	960
Colon I	1 in 2 dilution	1	1055	1037	1241
		2	1114	1108	1177
Colon II	Nil	1	878	831	930
		2	849	907	966
Colon III	1 in 5 dilution	1	1390	1290	1418
		2	1378	1290	1369
Colon IV	1 in 5 dilution	1	500	523	595
		2	482	450	654
Small colon anterior	Nil	1	449	421	—
		2	437	400	—
Small colon posterior	2 in 1 conc.	1	478	500	—
		2	493	483	—
Faeces	Nil	1	470	535	845
		2	450	594	861

Table 4. *Results of counts on material from horse no. 8 showing comparisons between four jar samples, A, B, C and D*

Horse no. 8	Concentration of counting suspension	Tube	Sample *A*	Sample *B*	Sample *C*	Sample *D*
Caecum	Nil	1	610	774	480	554
		2	575	815	445	580
Colon I	1 in 4 dilution	1	915	956	817	618
		2	886	996	952	582
Colon II	1 in 2 dilution	1	974	1220	861	991
		2	1032	1145	872	939
Colon III	1 in 4 dilution	1	1084	899	866	1668
		2	1005	829	848	1750
Colon IV	1 in 4 dilution	1	898	1245	1106	1043
		2	857	1158	1080	1100
Small colon anterior	Nil	1	544	—	—	—
		2	620	—	—	—
Small colon posterior	1 in 2 dilution	1	691	—	—	—
		2	687	—	—	—
Faeces	1 in 2 dilution	1	831	749	—	—
		2	783	727	—	—

(3) *Efficiency of the sieving for the separation of ciliates*

This was estimated by washing the residue a second and a third time. The number of ciliates found in the second wash was generally less than 5% of the total number recovered, and in the third wash, less than 1%. Finally, the residue on the sieve was examined microscopically: a small number of ciliates

could always be seen adhering to plant debris, but the number not removed by washing was negligible relative to the large number recovered in the first wash.

Table 5 shows the number of ciliates in each region of the gut of sample A_1, horse no. 7. The numbers recovered in the second wash are shown as a percentage of the total recovery and are less than twice the standard error of the first wash except in colon II, where the second wash number exceeded the standard error of the first wash by 0·6%. It was considered sufficient to sieve and wash the digesta only once, as figures so obtained represent at least 90% of the number actually found in any 10 g. sample. The percentage recovery was lowest in the faeces where the bulk of food residue was greatest.

Table 5. *The percentage recovery of ciliates in the second wash on all samples of A_1, horse 7*

	No. of ciliates per g. of gut contents	No. of ciliates in second wash, shown as a percentage of the total recovery
Caecum	$63{\cdot}6 \times 10^3 \pm 6{\cdot}8\%$	$3{\cdot}85 \pm 24{\cdot}2\%$
Colon I	$158{\cdot}25 \times 10^3 \pm 6{\cdot}1\%$	$4{\cdot}5 \pm 14{\cdot}1\%$
Colon II	$65{\cdot}85 \times 10^3 \pm 6{\cdot}7\%$	$6{\cdot}2 \pm 18{\cdot}5\%$
Colon III	$521{\cdot}25 \times 10^3 \pm 5{\cdot}4\%$	$2{\cdot}9 \pm 9{\cdot}7\%$
Colon IV	$187{\cdot}5 \times 10^3 \pm 8{\cdot}9\%$	$3{\cdot}4 \pm 15{\cdot}0\%$
Small colon anterior	$33{\cdot}67 \times 10^3 \pm 9{\cdot}4\%$	$4{\cdot}2 \pm 32{\cdot}0\%$
Small colon posterior	$17{\cdot}92 \times 10^3 \pm 9{\cdot}1\%$	$5{\cdot}1 \pm 37{\cdot}1\%$
Faeces	$35{\cdot}25 \times 10^3 \pm 9{\cdot}2\%$	$7{\cdot}7 \pm 22{\cdot}6\%$

RESULTS

(1) *Number distribution of ciliates*

The number distribution of ciliates in four starved horses is shown in Fig. 2. The ruled slide procedure was used to obtain these results, and the figures represent a mean of three counts.

Fig. 3 and 4 show the number distribution of ciliates in horses nos. 7 and 8 which were known to have been feeding up to the time of killing. Horse no. 7 was a Clydesdale, horse no. 8 a pony which had been fed on a diet of hay for 1 month previous to killing.

The Sedgwick-Rafter cell technique was used to obtain these results, and the histograms were constructed from the counts of tubes 1, shown in Tables 3 and 4. Each count was multiplied by the factor 300/4 plus the dilution factor in order to express the results as the number of ciliates per g. of gut contents in each subdivision of the large intestine.

For clarity in the diagram (Fig. 4), *A* represents the lowest count, *B* the second lowest, *C* the third lowest and *D* the highest of the four results obtained for each segment of the gut. The variation between samples from the same part of the gut may be considerable, but this variation does not mask the greater differences in the concentrations of ciliates from different parts of the large intestine. There appears to be a distribution pattern which is seen in starved horses nos. 3 and 4 and feeding horses nos. 7 and 8, showing a low concentration of ciliates in the caecum, a rise in colon I, and the highest concentration in colon III. The varying amounts of water in the gut contents do not appear to have any direct connexion with the concentrations of ciliates in the caecum and large colon, but the fall in water content of the food residue in the small colon and rectum probably does account for the rise in the numbers of ciliates in these parts (Fig. 4), since a greater volume of material is required to weigh out 10 g. of faeces.

(2) *Species distribution of ciliates*

Hsiung's monograph was used for identification of species; if there was any doubt as to the correct identification of a species a query has been inserted.

Table 6 shows the species distribution of ciliates in the different regions of the large intestine of five horses. It will be seen that the list of species may be divided into two parts:

(*a*) those species which are characteristic of the caecum, colons I and II, and occur only occasionally in the posterior portion of the large intestine;

(*b*) those species which appear in colon III and are characteristic of the posterior portion of the large intestine. In examination of material, the change in populations between colon II and colon III was very striking.

Blepharcorys uncinata was the most typical species of the caecum, where it occurred in greatest concentrations, its concentration being markedly less in colons I and II. Species of the genus *Cycloposthium* were predominant in colons I and II, *C. bipalmatum* being most regularly present. *Bundleia postciliata*, *Blepharocorys curvigula* and *B. angusta*? were the predominant species from colon III to the rectum. There did not appear to be so much fluctuation in the relative proportions of the species in the posterior part of the large intestine as in the anterior part. The empty skeletons of *Cycloposthium* species were of

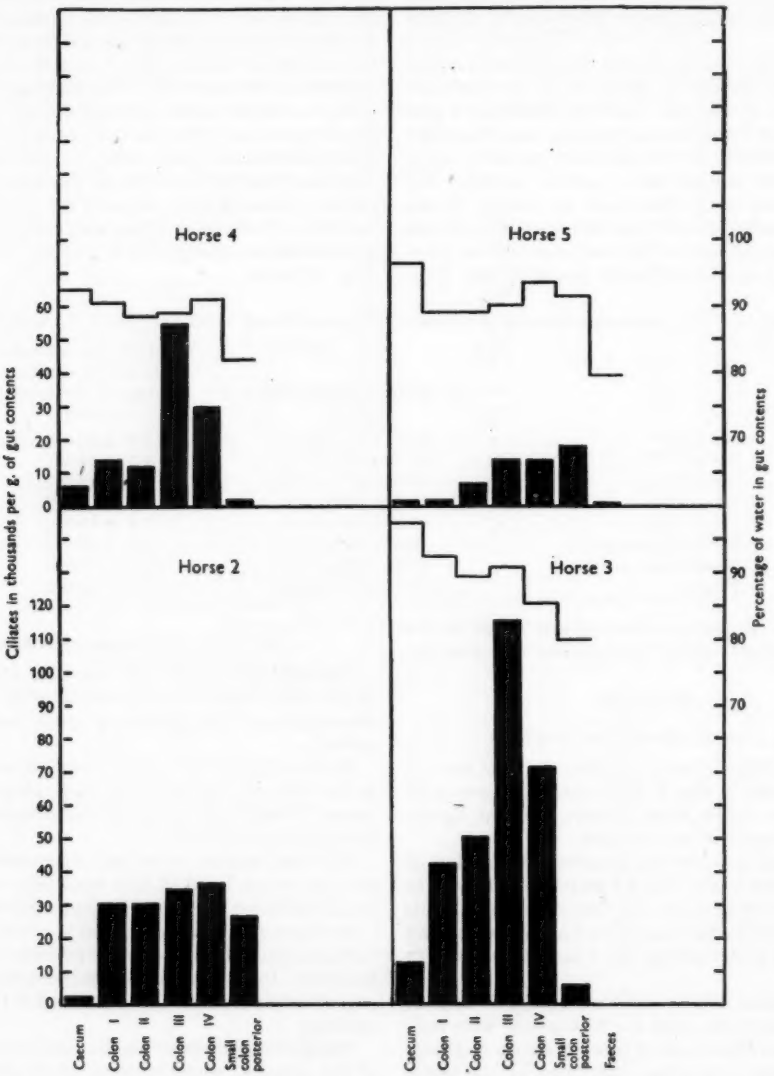

Fig. 2. Number distribution of ciliates in the different regions of the large intestine of starved horses, nos. 2–5. The percentage of water in the gut contents is shown by line above columns.

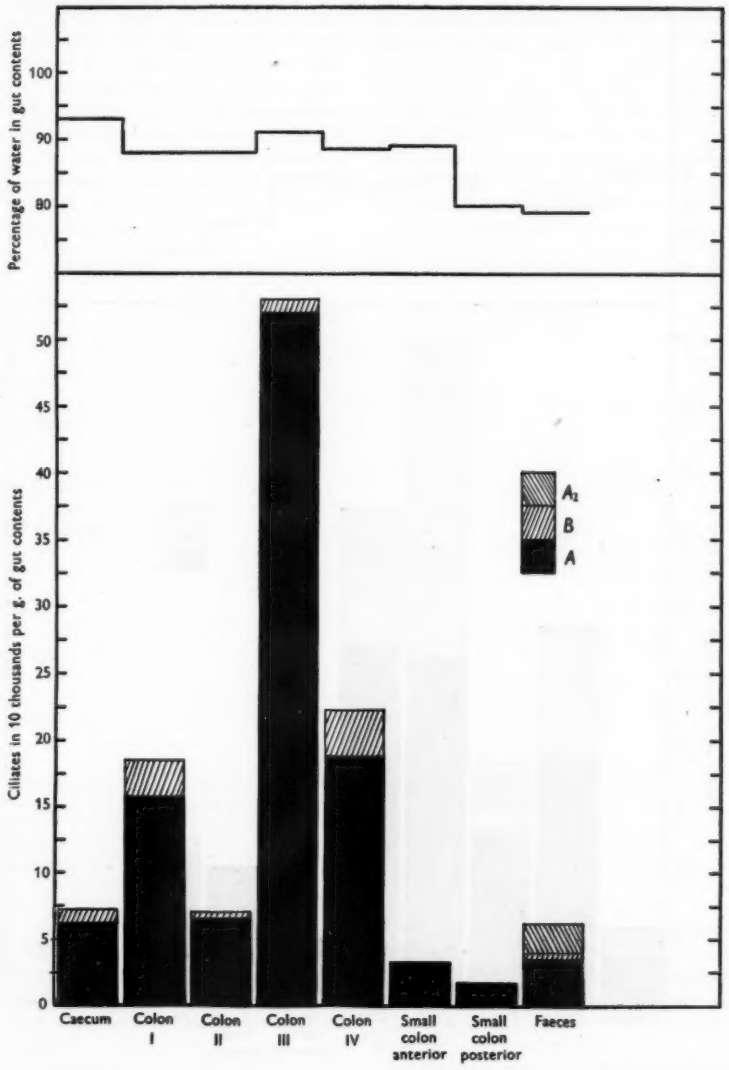

Fig. 3. Number distribution of ciliates in the different regions of the large intestine of horse no. 7. *A* and *B* represent results of counts on two separate 10 g. samples taken from each region of the gut.

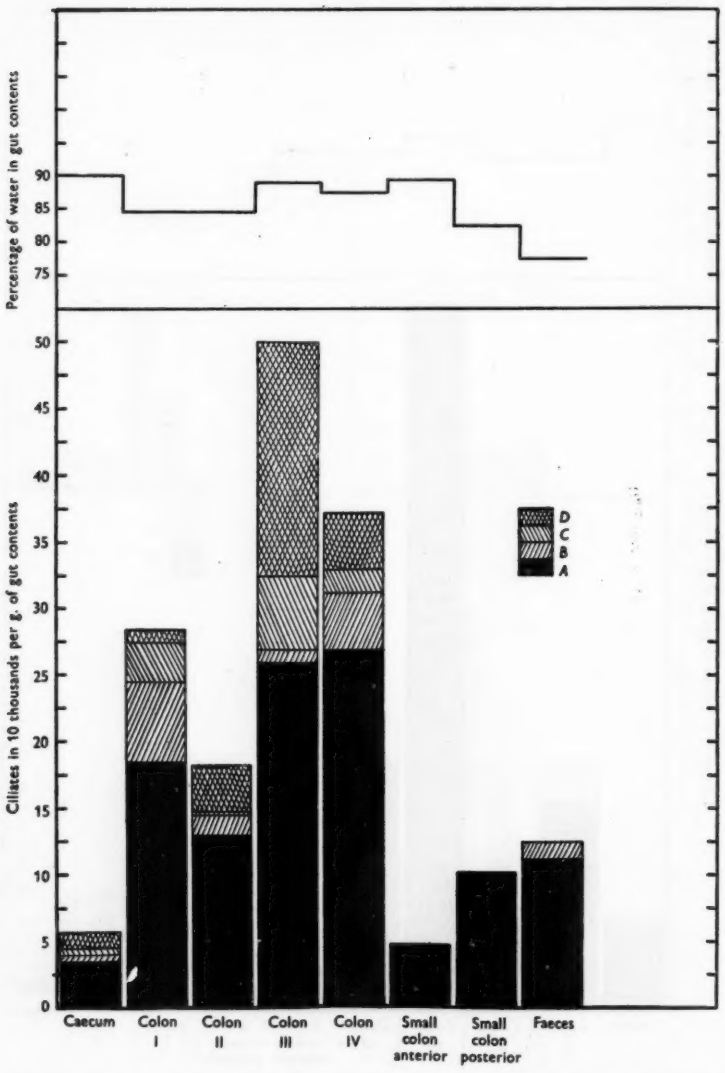

Fig. 4. Number distribution of ciliates in the different regions of the large intestine of horse no. 8. *A*, *B*, *C* and *D* represent results of counts on four separate samples of 10 g. taken from each region of the gut.

fairly common occurrence in material from the posterior part of the large intestine.

Although the total concentration of ciliates was distinctly greater in colon III than in colon I, the bulk was probably not so different as the predominant species of *Cycloposthium* in colon I were much greater in size. *C. bipalmatum* and *C. edentatum* have mean lengths of 101 and 190 μ and widths of 42 and 80 μ respectively (Hsiung 1930), whereas *Bundleia postciliata*, *Blepharocorys curvigula* and *B. angusta* have mean lengths of 42, 70 and 87 μ and mean widths of 25, 22 and 32 μ respectively.

DISCUSSION

It has been shown that the large intestine of the horse harbours a large population of ciliate Protozoa. The concentrations of ciliates varied in different regions of the large intestine, the highest concentration being found in colon III. Further, there were two distinct populations of ciliates, the first occurring in the caecum, colons I and II, and disappearing at the pelvic flexure where the second population appeared and persisted to the end of the gut.

As the protozoal population changes so does the appearance of the gut contents. In the anterior part of the large intestine the particles are coarse and green-brown in colour, in the posterior part the contents are more finely divided, a deeper olive-brown and the fluid is more dense. Further, the fluid content of the digesta rises in colon III.

Ciliates are typically animals of infusions, as their earlier name, the Infusoria, implies, and free-living forms are abundant in water containing a high concentration of organic material and large numbers of bacteria. The majority of ciliates are adapted for feeding on minute particles which are swept into the cell body in a current set up by the activity of a group of feeding cilia. The ruminant stomach and the large intestine of the horse present suitable media for the growth of ciliates. It is likely that the ciliates which inhabit the alimentary canal of herbivorous mammals

Table 6. *Species distribution of ciliates in five horses*

	Caecum	Colon I	Colon II	Colon III	Colon IV	Small colon and rectum
Didesmis ovalis	× × × × ×	× × × × ×	× × × × ×	× ×	×	× ×
D. quadrata	× × × ×	× × × ×	× × × ×	×	—	—
Blepharozoum trizonum	—	×	×	—	—	—
Blepharoprosthium ampulla?	× ×	×	× ×	× ×	× ×	×
Paraisotricha colpoidea	× ×	× × ×	× ×	×	—	—
P. beckeri	×	×	×	—	—	—
P. minuta	× × ×	× × × × ×	× × × × ×	×	×	×
Blepharocorys uncinata	× × × ×	× × × ×	× × × ×	×	×	×
B. jubata	×	×	×	×	—	—
Cycloposthium bipalmatum	× × × ×	× × × ×	× × × ×	× × ×	×	×
C. dentiferum	—	×	×	×	—	—
C. edentatum	× × ×	× × ×	× × ×	× ×	× ×	× ×
Unidentified sp.	×	×	×	—	—	—
Bundleia benbrooki?	—	—	—	×	×	—
B. postciliata	×	×	× ×	× × × × ×	× × × × ×	× × × × ×
Blepharocorys curvigula	—	—	—	× × × × ×	× × × × ×	× × × ×
B. angusta?	—	—	×	× × ×	× × ×	× × ×
Spirodinium equi	—	—	—	× × ×	× × ×	× × ×
Triadinium caudatum	—	—	×	× × ×	× × ×	× × ×
T. galea	×	—	—	× × ×	× × ×	× × ×
T. minimum	—	—	—	× × ×	× × ×	× × ×
Tetratoxum excavatum	—	—	—	×	×	×
T. parvum	—	—	—	×	×	×
Tripalmaria dogieli	—	—	×	×	×	×
Ditoxum funinucleum?	—	—	—	× ×	× ×	× ×
Allantosoma intestinalis	—	—	—	× × ×	× × ×	× × ×

× denotes occurrence in a single horse.

(3) pH *of samples*

Tests carried out with B.D.H. Universal Indicator about 2 hr. after killing showed the pH of the digesta to be in the range 7–8 except for the faeces where it was in the range 6–7.

(4) *Water content of the digesta*

The mean values for the percentage of water in the digesta of six horses were as follows: caecum, 93·0 %; colon I, 89·9 %; colon II, 88·7 %; colon III, 90·5 %; colon IV, 89·7 %; small colon anterior, 90·0 %; small colon posterior, 82·8 %; faeces 78·7 %. It appears that some concentration of the digesta occurs in the region of colons I and II and, to a greater extent, in the small colon posterior where the faeces begin to be formed.

are commensal forms, and do not significantly harm or benefit their hosts.

The ciliates found in the large intestine of the horse present a wide variety of forms. The smaller and simpler types probably feed mainly on bacteria; the larger and more complex types, which often showed conspicuous pieces of plant material in their bodies, may be able to utilize vegetable debris. The fauna may therefore be regarded as a diverse population of animals with different feeding habits and physiological requirements. This view is supported by the abrupt change in the population of ciliates at the pelvic flexure and by the large difference in concentration of ciliates in the caecum and colon I. Colon I appeared to be particularly favourable to ciliate growth; dividing forms were conspicuous in material from this part of the gut.

Becker & Hsiung (1929) showed that transference of ciliates from host to host takes place through the saliva in ruminants. In the case of the horse, the faeces contained a large number of living and active ciliates but no cysts, hence infection probably takes place by contamination of food with active forms present in the faeces. However, since some authors have described cyst-like forms in certain equine ciliates, transmission by cysts cannot be entirely excluded. Rumen ciliates are subject to digestion *in vitro* by peptic and tryptic extracts (Baker, 1943), but in the horse some ciliates appear to escape digestion in their passage from mouth to caecum.

Many facts are now known about digestion in the ruminant (Baker & Harriss, 1947; Elsden & Phillipson, 1948), and it is likely that digestion in the large intestine of the horse resembles that in the forestomach of ruminants. Elsden, Hitchcock, Marshall & Phillipson (1945–6) have shown that volatile fatty acids similar to those produced in the rumen by bacteria, are present in the large intestine of the horse, and Baker & Martin (1939) have demonstrated the breakdown of cellulose by bacteria in the caecum of the horse.

Little is known about the passage of food through the horse gut. Alexander (1946), using carbon granules as a marker, showed that these appeared in the faeces 22·6 hr. after feeding and ceased to appear after 47·4 hr. Smith (1912) recorded that maize reached the pelvic flexure 4 hr. after feeding.

The retention of a large population of ciliates in the horse gut is a question of particular interest. When seen living under the microscope the ciliates are continuously active, but the digesta are so dense and the relative size of the ciliates is so small that it appears unlikely that the ciliates could effectively alter their position in the gut.

If it is assumed that the ciliates form a static population which is carried along passively with the food, then continual replacement of the population from an external source must occur. This is unlikely to happen since the concentration of ciliates in the faeces is low and ciliates are not likely to survive long outside the gut.

The long time interval in the appearance of markers in the faeces, and the varying concentrations of ciliates found in similar parts of the gut suggest that the movement of food in the gut is irregular. Food probably collects in the sacculations of the caecum and large colon, and antiperistaltic waves may further slow down its rate of passage. In this way antiperistalsis and food pockets would enable the Protozoa to attack oncoming food. This view implies a dynamic population with a high rate of multiplication; but dividing forms were never seen in great abundance, and it is believed that the process of division requires a considerable time for completion. Finally, passive movement of either static or dynamic populations means that large numbers of ciliates disintegrate at the pelvic flexure and at the beginning of the small colon.

On the other hand, it is possible that the ciliates are capable of directed movements and can maintain position in the gut in response to stimuli. This view is supported by the peculiar complexity of structure shown by the larger ciliates, which are among the most highly specialized Protozoa. Further, the smaller ciliates show structures in their cells named 'concretion vacuoles', which are peculiar to those ciliates which are found in the guts of mammals. Dogiel (1929) thought that the concretion vacuoles corresponded in function to the statocysts found in many invertebrates.

Direct comparison of the numbers of ciliates found in the horse gut with those found in the rumen is not satisfactory. No single or controlled method has been used for estimating the density of the rumen fauna (Mowry & Becker, 1930; Van der Wath & Myburgh, 1941; Johnson, Hamilton, Robinson & Garey, 1944). The numbers are based on small samples of fluid withdrawn from the rumen. Figures obtained in this way are not likely to be representative of the entire population since it has been shown that wide variations occur in small samples taken from the same part of the horse gut. 500,000 to 1,000,000 ciliates per ml. appears to be an average figure for the concentration of ciliates in ruminal fluid, whereas 500,000 per g. was the highest concentration found in the digesta of the horse. It is not possible to use a volume measurement for the horse digesta, which is semi-solid. Mowry & Becker (1930) estimated that the number of ciliates in the rumen liquor of goats fed on hay alone was about 200,000 per ml., but if starch and protein were added to the diet, the numbers increased to about 2,000,000 per ml. The horses used in this investigation showed no sign of grain in the digesta; it is therefore possible that much higher concentrations of ciliates would be found in horses fed on a mixture of hay and grain.

SUMMARY

1. A method is described for separating and enumerating the ciliate Protozoa which occur in the large intestine of the horse.

2. The concentration of ciliates varied in the different regions of the large intestine, being low in the caecum and highest in the left dorsal colon.

3. Two distinct populations of ciliates occurred in the large intestine of the horse, one from the caecum to the pelvic flexure and the other from the pelvic flexure to the rectum.

ADDENDUM

After the completion of this work, the attention of the writer was drawn to a monograph by Strelkow (1939) on the Infusoria from the intestine of Equidae.

In this work, Strelkow identified the ciliates in material from a large number of horses, and clearly showed the existence of two faunas in the horse gut, which he termed the proximal and the distal faunas. The proximal fauna inhabits the caecum and ventral colon; the distal fauna inhabits the dorsal and small colons; at the pelvic flexure the two faunas are mixed. In this respect, the writer's observations agree with those of Strelkow. Further, Strelkow found that the distal fauna contained more species (average 16·6 species per horse) than the proximal fauna (average 7·7 species per horse). He listed twenty-five species and forms for the proximal fauna, forty-three species and forms for the distal fauna, and seven species common to both faunas.

Strelkow also estimated the density of the ciliate population in different parts of the large intestine. His average figures for the number distribution of ciliates in twelve horses show a pattern similar to that found by the writer, but the concentrations are considerably higher; for example, his highest estimate was 2,990,000 ciliates per ml. as compared with 500,000 ciliates per g. recorded by the writer. This difference may be due to the method of sampling employed by Strelkow, who simply made a slit in the gut wall and squeezed out a portion of the gut fluid, which was then diluted for counting. Since the gut contents are semi-solid, it is difficult to see how a representative sample could be obtained by this method. He used the counting technique described by Dogiel & Fedorowa (1929). A similar method of counting the ciliates in 0·2 ml. of fluid on a ruled slide was abandoned by the writer because of its inaccuracy.

The writer used Hsiung's monograph (1930) for the identification of the ciliates, in which 51 species of Ciliata and Suctoria were described. Strelkow has revised the classification of the Ciliata from Equidae and added new species and forms. He describes a total of 87 species and forms, 79 of which occurred in horses (Russian). The remaining 8 species and forms were distributed as follows: 1 form in the donkey; 1 species in Chinese mules (Hsiung, 1935); 5 species and 1 form in a zebra shot in British East Africa. Where possible the writer has endeavoured to bring the nomenclature up to date by referring to Strelkow's classification.

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A NEW MONOSTOME CERCARIA FROM BITHYNOID SNAILS IN SINGAPORE

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(With 4 Figures in the Text)

INTRODUCTION

Work on larval trematodes has now become world-wide in scope. Investigations have been carried out in Europe, Asia, China, Japan, Africa, the Americas, as well as certain Pacific Islands and countries in the Near East. The writer has not, however, been able to find any record of work on the Malay Peninsula or the island of Singapore. The nearest country to Malaya in which investigations have been carried out is Indo-China, where Lagrange (1923) investigated the trematodes of that country.

During a visit to the Far East the writer had the opportunity of studying a cercarial infection in bithynoid snails collected on the island of Singapore. The cercaria was a monostome but had characteristics which were quite different from those monostomes so far recorded.

The following contribution gives an account of both the cercaria and the parthenita.

TECHNIQUE

All work was carried out on the living material. Cercariae and rediae were obtained by crushing the snail. All measurements were carried out on a glass slide under a cover-slip, water being withdrawn by means of a piece of filter-paper until active movement had ceased. Study of the salivary glands was made after staining with neutral red solution.

PERCENTAGE INFECTION

The species of snail was not identified, but in all, seventy-four snails were dissected. Of these 20% were infected.

PARTHENITAE

The parthenitae (Fig. 1) are rediae which are colourless, with the exception of the gut and the cercariae in them. The youngest rediae are 0·8 mm. long and the mature ones 4·0 mm., the lengths of the others being between these two extremes. All are broader posteriorly. No locomotor processes and no birth pores were seen. The pharynx is muscular and well developed, and is succeeded by a rhabdocoele gut which is very short and occupies a very small part of the body. Although many rediae were examined, none was found with developing daughter rediae, but all gave rise to cercariae, of which as many as fifteen to twenty were counted within one parthenita. All the cercariae are developed from germ balls situated at the posterior ends of the rediae. The excretory system (Fig. 2) is well developed and is best seen in the young redia. The excretory pore is centrally placed about one-third the distance from the anterior end, and it leads into a vesicle which was observed to contract and empty itself periodically. There arises from this vesicle a single large vessel which divides into an anterior and posterior tubule. The anterior one, which is coiled, passes forward and drains from a single flame cell near the posterior end of the pharynx. The other tubule passes posteriorly down the mid-line as a wide tube where, just behind the middle of the body, it bifurcates into two capillaries. One of these passes to the lateral region of the body, where, about one-third of the distance from the posterior end, it drains from a single flame cell; the other further divides into two branches, each of which passes to the lateral margin, where it is connected to a flame cell.

CERCARIAE

The cercariae (Fig. 3) swim continuously in the water and are extremely active. None was observed creeping along the bottom of the containing vessel. They are broadest at the posterior end of the body. Their length varies from 0·3 to 0·5 mm. Their breadth varies between 0·1 and 0·2 mm. according to the expansion or contraction of the body. The tail arises from a definite caudal pocket. When it is fully extended it is at least twice as long as the body. Its length varies between 1·1 and 1·3 mm. and its breadth between 0·03 and 0·05 mm. It has on both sides a number of setae which have a length approximately equal to half the width of the tail at the base. These setae extend along the whole length of the tail at irregular intervals.

On the anterior tip of the body is a row of four spines approximately 0·004 mm. in length, behind which is a second row of eight spines 0·003 mm. long. These two rows are followed by nine rows of very small spines, the number and length of which were not determined. The rest of the body has no spines.

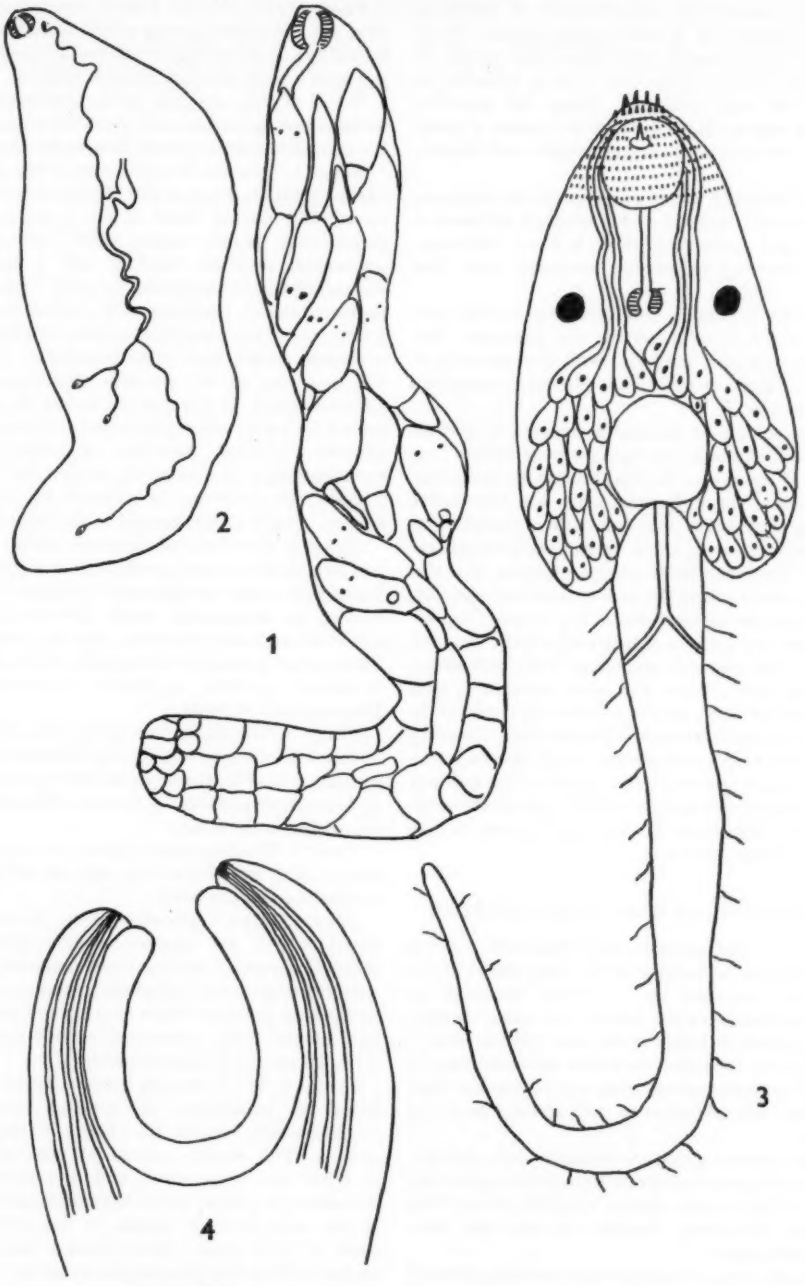

Fig. 1. Redia.
Fig. 2. Excretory system of redia.
Fig. 3. Cercaria.
Fig. 4. Cercaria, oral sucker and openings of salivary glands.

The oral sucker, the musculature of which is poorly developed, is a penetrating organ. It is capable of considerable protrusion and varies in length from 0·04 to 0·05 mm. with a breadth of between 0·03 and 0·04 mm. Near the anterior border is a stylet. It consists of two parts, a basal portion in which sits the needle, which is 0·05 mm. long.

Situated laterally about one-quarter the distance from the anterior end are a pair of highly pigmented eye spots. The diameter of these is about 0·02 mm. and they develop before the cercariae leave the rediae.

The mouth is ventral and leads into a long pre-pharynx which is followed by the pharynx. The pharynx is 0·03 mm. long and 0·02 mm. broad and is on a level with the eye spots. No other intestinal organs were seen.

The salivary gland apparatus consists of a large mass of cells, of which twenty-four were counted on each side of the body. It was difficult to make out the outlines of the individual cells, but the nuclei were quite prominent. This cell mass is posterior to the pharynx. From it three ducts pass forwards on each side. They run between the pharynx and the eye spots, curve round the oral sucker and open by a single aperture on each side of the mouth (Fig. 4).

The excretory system consists of a large bladder situated at the posterior end of the body. Its shape varies considerably, but was most commonly seen in the rounded form and it is believed that this is the shape assumed when it is full of fluid. Leading into the tail is a caudal tubule, which passes backwards for about one-tenth the length of the tail and then bifurcates into tubules which open on the sides of the tail. No trace of the other parts of the excretory system were seen.

IDENTIFICATION AND CLASSIFICATION

The presence of the caudal setae is distinctive and is in contradiction to Lühe's (1909) designation of the monostome cercariae as '...those cercariae in which an acetabulum was absent, eye spots present, the tail undivided, long slender and without setae'. This is the first time that the writer has seen setae on the tail of monostome cercariae and he has not been able to find any reference to such structures in the literature.

A large number of mature cercariae were studied, as well as the immature forms within the rediae, but no trace of the ventral sucker could be found. The writer has, therefore, decided to call this form *Cercaria setaecauda*.

The small group of monostome cercariae, defined by Lühe (1909) exclude forms with forked tails lacking an acetabulum and also those types which are akin to the Xiphidiocercariae which possess a stylet.

Faust (1917) divided Lühe's original group into two, the Binoculate group which have two distinct lateral eye spots and the other, the Trioculate group, in which a third median patch is present.

Sewell (1922) used the term 'monostome' in its widest sense to include all those cercariae in which the ventral sucker is absent. He suggested six groups.

Group 1. The Pleurolophocerca group characterized by their small size, a pair of pigmented eye spots, an anterior sucker more or less in the nature of a penetrating organ, many-celled salivary gland apparatus, reniform bladder and a tail with a cuticular fold. Development in redia. Dubois (1929), re-describing *C. lophocerca* Fil., found that, in the young cercaria in which the tail was still rudimentary, a ventral sucker was quite prominent, but during the maturity of the cercaria this organ became reduced, until, in the mature forms, it was represented by a depression just anterior to the excretory bladder. Dubois, therefore, suggested that the characteristics of this group should be such as to include the presence or absence of the ventral sucker. Sewell (1931) agreed to this emendation.

Group 2. The Urbanensis group, which is more or less Faust's Binoculate group in that it includes those forms with a pair of pigmented eye spots, a true oral sucker, an alimentary tract without a pharynx, spherical excretory bladder, with the main tubules uniting just posterior to the oral sucker, presence of locomotor pockets, a triclad alimentary canal. Development in redia.

Group 3. The Ephemera group, in effect Faust's Trioculate forms, including large cercariae, numerous cystogenous cells, three pigmented eye spots, a pair of locomotor pockets, a triclad alimentary canal. Development in redia.

Group 4. The Lophocerca group, fork-tailed monostomes with fin-folds on the rami as well as a longitudinal fold on the body.

Group 5. The Lophoides group, including other forms which are morphologically akin to the Xiphidiocercariae, its members possessing a stylet, a short pre-pharynx and pharynx but no oesophagus or caeca, a group of three to six gland cells on each side of the body, excretory vesicle rounded and a caudal excretory tubule present.

Group 6. The Ubiquita group. Apart, therefore, from its possession of distinct caudal setae, *C. setaecauda* cannot be placed in any of these groups. The simple nature of the tail excludes it from the Lophocerca and Lophoides groups. It cannot be placed in the Ephemera group because it has only two eye spots. It has affinities with each of the other three groups but must be excluded from the Pleurolophocercariae owing to its size, from the Urbanensis group because it has no locomotor pockets, and from the Ubiquita group because it develops in redia.

It must, therefore, remain unclassified.

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